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On the Eyes and Reactions to Light of Proteus anguinus.

By

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Department of Zoology, University College of the
South West of England, Exeter.

With 6 Text-figures and 5 Tables and 1 Plate.

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I. Introduction

Proteus anguinus Laurenti is a 'blind', unpigmented, neotenous Urodele, entirely confined to caves in Jugo-Slavia and Istria. The eyes are degenerate, but it has been known for a long time that the animals were exceedingly sensitive to light. Configliachi and Rusconi (1821), who kept specimens in captivity for two years, reported that the animal rests quietly on the bottom of its tank when in the dark, 'but if the vessel be quickly uncovered, he suddenly begins to move, is much agitated and seeks always that part of the vessel which is darkest'. The general effect of light as a disturbing influence was noted by v. Chauvin (1883) and Kammerer (1912).

Configliachi and Rusconi knew that Proteus had eyes of some sort, but they ascribed its photosensitivity to the skin. Joseph (1876, quoted by Kohl, 1892) thought that the eye was capable of orientating the animal. Hess (1889), Schlampp (1892), and Kohl (1892), who investigated the structure of the eye, all supposed that it was in some way functional, Schlampp even describing a specially transparent patch of skin which was supposed to transmit light to the eye; Kohl accepted the description and its alleged function. Schlampp further interpreted a supposedly pigment-free region of the retina round the anterior pole of the eye as the functional equivalent of the pupil in a fully developed eye. The only serious experimental investigations on the subject were those of Dubois (1890); his results supported the views of the histologists and have been generally accepted. He illuminated normal animals and those whose eyes had been covered by a mixture of lamp-black and glycerine and found that the former reacted twice as quickly to light as the latter; from these and other experiments he concluded that both eyes and skin were sensitive to light and that the sensitivity of the eyes was twice that of the skin.

In 1908, Hirsch-Tabor, in a little known paper, announced that the optic tracts of the mid-brain were missing, that there was no optic chiasma and that the optic nerve existed only within the bulbus oculi and as a short stump outside it and failed to reach the brain. Unfortunately, his note on the optic nerve was based on sections of only one specimen. The more recent work of Kreht (1931) confirmed that the optic system of the thalamus was absent but he does not mention the optic nerve; there is nothing to represent it in his figure of a reconstruction of the brain and this suggests that he failed to find the nerve.

The position was, then, that all observers agreed that Proteus was sensitive to light and most of them considered that the eye played some part in light perception; this view was supported by the only available experimental evidence. On the other hand, anatomical work suggested that the optic nerve, at least in the adult, might not reach the brain. In addition, it is doubtful if any modern investigator would accept the methods (discussed below) employed by Dubois. Apart from the obvious need for both anatomical and experimental reinvestigation here, there does not exist any precise description of the light reaction of Proteus, and there is nothing, beyond speculation, about the role of its photosensitivity in the animal's life in its native caves. In the course of the anatomical investigation on the optic nerve the opportunity was taken to check previous descriptions of the eye itself and to describe such accessory features as the vascular supply and the extrinsic eye muscles. This communication therefore deals with the following:

The anatomy of the orbit, especially in relation to the eye and its accessory organs.

The parts played by eyes and skin in light perception.

The nature of the animal's reactions to light and consideration of the part played by them in nature, based on investigations made in Jugo-Slavian caves and in the laboratory.

The account of the results falls naturally into two parts, anatomical and experimental, and the methods used in each are described separately.

II. ANATOMICAL INVESTIGATIONS.

1. Material and Methods.

The anatomical descriptions are based on examinations of serial sections, cut transversely and longitudinally, of the heads of eight animals; the smallest was 8·4 cm. and the largest 34·5 cm. long from the snout to the tip of the tail. After decapitation, the heads were fixed in Zenker-formol-acetic, Duboscq-Brasil's fluid or corrosive acetic (95 c.c. sat. aq. soln. mercuric chloride+5 c.c. glacial acetic acid), decalcified, dehydrated and cleared in cedar-wood oil or toluol.

One series was embedded in paraffin wax; some were double embedded, in 2 per cent. celloidin followed by paraffin wax. While these methods were useful for reconstructing the skull and other large structures, celloidin was employed for the study of fine detail, e.g. following the scanty threads of muscle composing the extrinsic eye muscles. The material was placed in 2 per cent. celloidin and the solution gradually thickened over two to three months until it was approximately 20 per cent. After hardening in chloroform and shaping the blocks, they were strengthened by impregnation with paraffin wax (MP = 45°C.). Such blocks can be cut with a sledge microtome into serial sections at 8–10 μ, disturbance of the most delicate structures is reduced to a minimum and the staining reactions of the material are greatly enhanced. I am much indebted to Mr. K. C. Richardson for introducing me to this technique and for allowing me facilities to apply it in his laboratory at University College, London.

2. General Structure of the Orbit.

The orbit is bounded mesially by the parietal bone and the trabecula cranii, anteriorly by the processus antorbitalis, and posteriorly by the processus ascendens quadrati; the pterygoid bone juts obliquely outwards from below the trabecula and forms a floor, broader posteriorly than anteriorly, to the orbit. The form of these structures in this region is as follows. (Text-figs. 1 and 2; Pl. 1.)

The parietal (*P*) forms the roof of the skull posteriorly, meeting the frontal, which overlaps it, in a transverse suture on top of the head just anterior to the auditory capsule. From its horizontal region, behind the frontal, it sends downwards a vertical sheet of bone, which is continued forwards as the wall of the orbit. This orbital process of the parietal articulates above with the lateral edge of the frontal and rests below on the parasphenoid (*Psph*). For most of its length it bears a horizontal flange jutting outwards above the trabecula and, in places, bending over it (Text-fig. 2). Posteriorly, at the anterior level of the auditory capsule, the margin of the orbital process is notched to accommodate the Gasserian ganglion. Somewhat anteriorly to this, it bears a slight process which meets the ascending process of the quadrate; a little further forward the bone is penetrated by a small aperture, the foramen opticum, through which the optic nerve and ophthalmic artery enter the orbit from the cranial cavity. In my smallest animal the foramen was 0·165 mm. long and 0·27 mm. high, but the trabecula lies so close to the parietal in this region that it partly occludes the foramen, so that only a tiny crack, 0·06 mm. high and 0·1 mm. long, is left for the passage of the nerve and artery; the foramen is always minute.

The trabeculae cranii (*T*) are paired rods lying in the angle formed by the lower part of the parietal wall of the orbit and the outer edge of the parasphenoid, which projects laterally somewhat beyond the parietal. Each trabecula runs forwards through the entire orbit. It is entirely cartilaginous in this region in young animals, but is lightly ossified anterior to the antorbital process. In older animals this ossification may spread backwards along the mesial surface of the trabecula as far as the level of the eye.

Just anterior to the eye and behind the internal naris lies the processus antorbitalis (*Prant*). It is a large cartilage, curving outwards and forwards, not, as in the illustrations by Parker (1877) and Wiedersheim (1877), outwards and backwards; according to Winslow (1898), the former is the normal condition in Urodela. Its fusion to the trabecula is feeble in young animals and, though it is stronger in older ones, the junction is never completely chondrified.

The quadrate (*Q*) is a large, partially ossified cartilage attached to the skull by its processi oticus and ascendens and by the squamosal. Ossification begins on the lateral side of the otic process, extends downwards and forwards to encompass the entire body of the quadrate as it approaches the mandible, but leaves the cartilage exposed in the area of the articulation.

The otic process (*Prot*) is directed backwards from the dorsal part of the body of the quadrate towards the auditory capsule and ends between the dorsal process of the squamosal plastered on to the capsule and the most anterior part of the pro-otic. Parker noted that the otic process ended freely in Proteus. This is rather misleading, especially when taken in conjunction with his statement quoted below. It is true that there is no fusion between the process and the capsule, but the position of the former, wedged into the notch between the capsule on the inside and the squamosal on the outside, must make a firm but slightly movable support against any backward thrust which may be transmitted from the mandible.

According to Parker 'the suspensorium has only one junctional process at its apex, the pedicle, which is fused with the trabecula below'. The pedicle of Parker and Huxley is the processus basalis of modern authors. I am unable to find in any animal any process of the quadrate which lies between the ramus palatinus facialis and the vena capita lateralis or which fuses with the trabecula, and conclude that the basal process is absent.

The processus ascendens (*Prasc*), on the other hand, is present. It arises anteriorly to the auditory capsule as a broad cartilaginous projection passing upwards and inwards to join a bony protuberance on the parietal. It lies dorso-lateral to the vena capita lateralis and the ramus ophthalmicus profundus trigemini and ventro-mesial to the rami maxillaris and mandibularis of that nerve. Its relations in Proteus are those regularly found in Urodela and there can be no doubt about its identity.

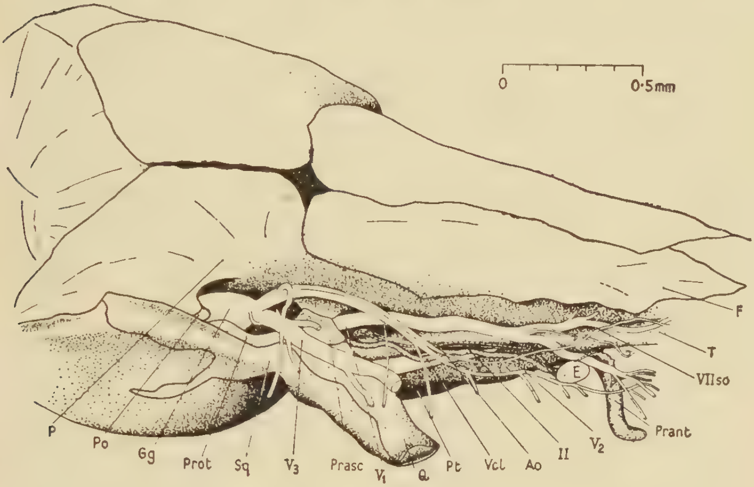
The quadrate is further supported by the squamosal (*Sq*), and, to some extent, the pterygoid (*Pt*). The former bone lies for the most part over the auditory capsule, but sends downwards and forwards a long process which overlies the outer face of the quadrate almost as far as the articulation with the mandible. The pterygoid is in contact posteriorly with the mesial face of the quadrate, but for a shorter distance than the squamosal; these two bones grip the quadrate in a secure but not rigid clasp.

Almost the whole of the available space in the orbit is occupied by the M. levatores mandibulae, and the remaining organs are crowded among them; anterior, exterior, and posterior levators can be distinguished.

The Gasserian ganglion lies partly within a recess formed by the mesial wall of the auditory capsule and otic process of the quadrate on the outside and the parietal bone above and on the inside, and partly exposed in the orbit itself. From it originate the three main branches of the trigeminal nerve and the superior ophthalmic branch of the facial. For present purposes the relations of these nerves are sufficiently evident from Text-figs. 1 and 2 and the figures in Pl. 1.

3. The Eye and its Accessory Organs.

The eye (Fig. 1, Pl. 1) is just posterior to the processus antorbitalis, between the superior ophthalmic, maxillary, and lateral ramus of the profundus nerves, which are respectively dorsal, ventral, and mesial to it, and may even be in contact with it. It is generally visible in the living animal as a pigmented spot about the size of a pin's head. According to most authors, the eyes lie at the posterior ends of paired grooves on the dorsal surface of the head, but I have found these grooves very inconstant in their occurrence, especially in living animals.



Text-fig. 1.

Graphical reconstruction of the orbit of Proteus anguinus (8·4 cm.). *Ao*, arteria ophthalmica; *E*, eye; *F*, frontal; *Gg*, Gasserian ganglion; *P*, parietal; *Po*, pro-otic; *Prant*, processus antorbitalis; *Prasc*, processus ascendens quadrati; *Prot*, processus oticus quadrati; *Pt*, pterygoid; *Q*, quadrate; *Sq*, squamosal; *T*, trabecula cranii; *Vcl*, vena capita lateralis; *II*, nervus opticus; V_1, V_2, V_3, rami ophthalmicus profundus, maxillaris et mandibularis trigemini; *VIIso*, ramus ophthalmicus superficialis facialis.

They often appear in fixed material, or in animals kept for a long time without food, but in healthy well-fed specimens the snout is usually quite smooth. The eye is immovably embedded in the tissue about it, i.e. in loose connective tissue or fat or both; the fact that the amount of subcutaneous fat varies from time to time led the earlier workers to dispute over the nature of the tissue about the eye.

In form the eye is somewhat variable, but ordinarily resembles a sphere which has been slightly contracted along the optical axis and somewhat elongated in a cranio-caudal direction. The only important fact which emerges from measurements of individual eyes is that a considerable increase in size takes place after differentiation stops. In the following pairs of figures, the first is the length of the animal in centimetres and the second is the length of the optical axis in microns: 8·4, 225; 13·0, 375; 17·3, 240; 20·6, 375; 22·1, 525; 23·8, 385; 26·5, 485; 34·5, 485.

The histology of the eye was described in detail by Schlampp in 1892, and by Kohl in the same and the following years. The description by Schlampp is so complete and accurate that I have very few corrections to make to it. Kohl's lengthy publication added nothing of importance to Schlampp's account; his excessively detailed descriptions of insignificant variations in the eyes of different individuals are so full of errors that it would be unprofitable to attempt to correct them all. The earlier literature was summarized at length by Kohl and there is no need to deal with it again. All of it was superseded by Schlampp's paper. As Hess (1889) had already recognized, Schlampp confirmed that the structure of the adult eye was the product of arrested development at the secondary optic vesicle stage, followed by the degeneration and loss of the lens.

The eye is surrounded by a delicate, collagenous sclera, continuous except at the entrance of the optic nerve and blood capillaries, and without a cornea.

In the larvae of Urodela generally, cartilaginous platelets occur in the sclera; they are absent from adults. Since their disappearance is normally associated with metamorphosis, the condition in Proteus is important. They were discovered by Leydig in 1857 and seen again by Desfosses in 1882; Hess (1889) was unable to find them, but Schlampp and Kohl (op. cit.) described them in some detail. Both the latter authors agreed that in young animals there was very little cartilage, but that chondrification increased with age. In the eight specimens examined I found cartilage cells in only two. In one (26·5 cm. long) there was a tiny platelet of cartilage in the extreme anterior face of the sclera; it consisted of about a dozen cells and was approximately 65 μ in diameter. In the other (23·8 cm. long) the plate was much larger and lay entirely in the posterior half of the sclera on the ventro-lateral aspect of the eyeball. It began just behind the middle of the eye as a thin wedge, increased to a height of about 200 μ and then narrowed and ended about 250 μ behind its starting-point. Anteriorly it was penetrated by a capillary from the choroid, and posteriorly it received the inferior rectus muscle. It is important to note that cartilage was entirely absent in animals larger and smaller than these two and that chondrification was much more extensive in the smaller animal. The scleral cartilages are inconstant in their occurrence and irregular in their size and position; there is no evidence that chondrification goes on after degeneration of the eye begins (as implied by Schlampp and Kohl) and, evidently, the presence or absence of cartilage here has nothing to do with the metamorphic agents producing neoteny in Proteus. The same is apparently true of Amblystoma, for here the scleral cartilages persist after metamorphosis, though they regularly disappear in other Urodela (Stadtmüller, 1929, quoted by de Beer, 1937).

The choroid is unremarkable and consists of a fibrous, pigmented supra-choroid, a capillary layer and a basal membrane, all accurately described by Schlampp.

The pigmented epithelium of the retina, 10–15 μ broad in fully grown animals, consists of a single layer of hexagonal cells tangentially flattened against the choroid, from which they are separated by the basal membrane. They show no signs of degeneration and no features of special interest. The conflicting statements of previous authors about the distribution of pigment are unreliable and were probably based on examination of too few specimens. Pigment granules are irregularly distributed throughout the epithelium, forming local accumulations in almost any region. The body pigment of Proteus is always lost, although animals kept in the light rapidly form it. In the pigmented epithelium some granules are always present. It was exceedingly dense throughout the epithelium of both my small animals, and always more sparse in older ones, which suggests that pigment is not produced throughout life in the epithelium, but does not degenerate, so that the young animal's store of pigment is distributed over a wider area in older animals, in which, therefore, individual cells are less densely crowded with the granules. In a few animals slight cytoplasmic processes containing pigment were pushed out towards the visual elements of the retina, but mostly the pigment cells presented a flat surface towards the interior of the eye and the pigment was located in the body of the cell.

All authors agree that the usual layers of the retina can be distinguished in the eye of Proteus. Ganglionic fibres and cells are easily recognized, though their outlines may be contorted by the pressure of the ingrowing cell mass from the 'iris'. The inner plexiform layer is well defined and wide. The remaining inner and outer nuclear and outer plexiform layers can be recognized, though the arrangement of their elements is less strikingly regular than in the fully developed urodele eye.

The visual cells of Proteus are always small and somewhat variable in structure. These elements in Urodela are characterized by the presence in their inner segments of a well developed lenticular apparatus, with a granular ellipsoid, staining pink in orange G-erythrosin, and a clear paraboloid, staining pale yellow. Proteus eyes, stained with larval Salamandra eyes as controls, showed a few visual elements in which the lenticular bodies were already differentiated; more generally, the element consisted of a short, more or less conical, projection above the nucleus, with a mass of deeply staining granules at its base. According to Detwiler and Laurens (1921) this is the appearance of the embryonic urodelan visual element, just before differentiation of the lenticular apparatus; indeed, most of the visual cells of Proteus might well be represented by their Figure 2 of early rod and cone primordia in Amblystoma. Previous authors have failed to distinguish between rods and cones in Proteus, but Detwiler and Laurens (op. cit.) have shown that these elements in Amblystoma can be separated at a very early stage. In Proteus there are some elements with large nuclei projecting slightly beyond the external limiting membrane, and often with their outer segments ending bluntly and showing some differentiation of ellipsoid and paraboloid. They resemble the rods of Amblystoma from four to six days after their appearance. There are also elements with somewhat smaller nuclei, lying below the membrane, and usually with rounded or conical ends. They are rudimentary cones. But between these lie all intermediate types, and some which are too degenerate to allow classification. The state of this layer is best accounted for on the assumption that development is stopped at about the stage when rods and cones are beginning to acquire their distinctive features, usually before the lenticular apparatus is fully differentiated, and this arrest is followed by degeneration of the distal parts of some visual elements.

There is always an external and usually an internal limiting membrane, and between the two the supporting fibres of the retina run. They can plainly be seen ending in the membranes, and Kohl's figures (1892, Pls. V and VI), showing Müller's fibres reaching the pigment epithelium, are inaccurate.

Schlampp was the first to give a satisfactory account of the history of the lens in Proteus. According to him the rudiment of the lens is present in the larva; it does not differentiate fully, but begins to degenerate; it is still present, though reduced, in young adult animals (10–12 cm.), but is completely absent in older individuals. Its place is taken by a cellular mass proliferated from the borders of the retina and pigmented epithelium where they would form the iris of a normal eye. His statement is true in general, but the condition is more variable than he supposed. In my two smallest specimens (8·4 and 13 cm.) there is no trace of a lens and the border of the iris has already begun its irregular inward growth. But in two much older animals (20·6 and 22·1 cm.) the lens is still present and does not differ significantly in structure from that of the larval eye described by Schlampp. The lens is a ball of tightly packed cells with large nuclei surrounded by a collagenous capsule. In both cases the presence of the lens had inhibited the proliferation of cells at the borders of the 'iris' which is typical of eyes without lenses.

Schlampp denied that a vitreous body was present in Proteus at all, but Kohl claimed to have found one feebly developed and consisting mainly of connective-tissue fibres and cells. In the two large specimens of mine in which the lens persisted the posterior chamber of the eye was also preserved. In each case this contained some branching fibres which stained like collagen in Masson preparations. They were derived from both retinal and lenticular ('ciliary') borders of the chamber and some were continuous with the capsule of the lens. There were also a few cells resembling leucocytes, and possibly phagocytic. Such fibres probably represent the remains of a vitreous body which begins to degenerate before completing its differentiation. In other specimens a few fibres with similar staining reactions occurred among the cell mass which filled the cavity after the lens had degenerated.

The nervus opticus (Text-figs. 1 and 2; Pl. 1) leaves the eye ventrally and, usually, somewhat posteriorly. It passes downwards and backwards, in company with the ophthalmic artery, behind the lateral terminal branch of the deep ophthalmic, below the anterior levator of the mandible and then decreases in diameter to a thread, 15–20 μ in diameter. It runs backwards, laterally to the trabecula cranii, mesially to the levatores mandibulae, dorsally to the vena capita lateralis and ventrally to the nervus ophthalmicus profundus, and enters the cranial cavity, in company with the ophthalmic artery, by the foramen opticum. Here it runs backwards and inwards, parallel and mesial to the artery, to enter the floor of the diencephalon near to its anterior limit (Fig. 2, Pl. 1).

The measurements on p. 13 show that the nerve grows for some time after development of the eye stops.

The embryonic lumen of the optic vesicle is retained in the optic nerve of some larval urodeles and even adults; e.g.

Necturus (Kingsbury, 1895), where the nerve is hollow distally. In Proteus it is solid throughout its length. For most of its course it is surrounded by a collagenous sheath, continuous with the sclera where the nerve leaves the eyeball.

Length of animal	*Diameter of optic nerve*	
	near eye	*near f. opticum*
8·4 cm.	20 microns	10 microns
13·0 ,,	25 ,,	12 ,,
17·3 ,,	45 ,,	15 ,,
20·6 ,,	35 ,,	20 ,,
22·1 ,,	60 ,,	15 ,,
23·8 ,,	45 ,,	15 ,,
26·5 ,,	60 ,,	35 ,,
34·5 ,,	45 ,,	15 ,,

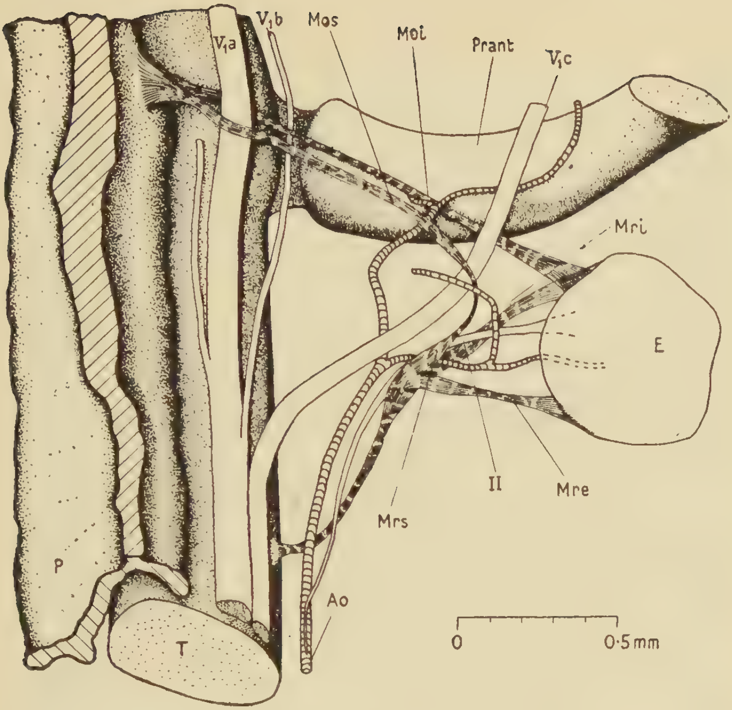
Schlampp noted the presence of blood in the choroid, but the vascular supply of the eye has not previously been investigated. It is greatly reduced from the normal amphibian condition, but the ophthalmic artery is present and retains its essential relations throughout life (Text-figs. 1 and 2; Pl. 1). The internal carotid artery passes diagonally inwards below the auditory capsule, gives off a small branch to the palate and enters the cranial cavity, close to the anterior border of the capsule and between the floor of the latter and the parasphenoid bone. Within the skull it turns forwards and immediately gives off a large branch (cerebral artery) to the brain. From this point the vessel is minute and may be regarded as the ophthalmic artery. It turns outwards, enters the orbit through the foramen opticum and there follows the course of the optic nerve. It turns outwards with the latter and, in very young animals (8–13 cm.), it passes the eye in close contact with it; in all larger individuals it sends a branch to the eye which penetrates the choroid obliquely. The rest of the vessel branches, contributing to the orbital capillary network, supplying the extrinsic eye muscles, and then passing forwards above the processus antorbitalis. For most of its course this vessel was 20–25 μ in diameter in a young animal (8·4 cm.), and only 30–40 μ in fully grown specimens. Yet, in spite of its capillary dimensions, it must be regarded as truly representing the ophthalmic artery.

The vena capita lateralis enters the orbit below the processus ascendens. In young animals it receives no factors in the orbit itself, but arises from a number of small branches dorsal to the processus antorbitalis. Like the artery, it lies in close contact with the eyeball. In older animals blood leaves the choroid in a capillary which joins the vascular network in the anterior part of the orbit: some of its components, at least, drain into the vena capita lateralis, which persists in its primitive form throughout life.

Kohl (1892) mentioned that all the extrinsic eye muscles were present, but he did not describe them, while both Hess (1889) and Kammerer (1912) asserted that all were absent. I have examined these muscles from celloidin sections of four fully adult specimens of Proteus. There are always some muscles present and they are always greatly reduced in size. As the eye is embedded in the surrounding tissue, they must be functionless, and they give way to and curl round the larger structures in their paths. But, histologically, they show no signs of degeneration and their elements are indistinguishable in structure and staining reactions from the fibres of such functional muscles as the levators of the mandible.

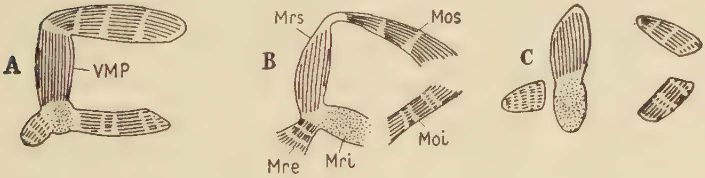
The small size of the eyeball and peculiarities in the arrangement of the muscles sometimes make it difficult to identify the latter from the relative positions of their insertions and origins, and it is therefore necessary to rely on their anatomical relations and comparison with the embryonic condition in other urodeles as far as that is known. As the condition of these muscles is of some importance in relation to a point raised below, I shall describe the condition in detail in one specimen (26·5 cm.) and briefly discuss the differences seen in the others.

In the specimen illustrated in Text-fig. 2 and fig. 1, Pl. 1, just behind the eye, a thin strand of striated muscle (15–20 μ in diameter) arises from the trabecula cranii and passes outwards below the deep ophthalmic and optic nerves, the ophthalmic artery and the vena capita lateralis. It then runs forwards parallel with these structures and turns outwards, with the



TEXT-FIG. 2.

Graphical reconstruction of part of the orbit of Proteus anguinus, seen from above. The branches of the fifth nerve are reduced in size and somewhat diagrammatic. *Ao*, arteria ophthalmica; *E*, eye; *Moi*, *Mos*, musculi obliqui inferior, et superior; *Mre*, *Mri*, *Mrs*, musculi recti externus, inferior, et superior; *P*, parietal; *Prant*, processus antorbitalis; *T*, trabecula cranii; *II*, nervus opticus; V_1a, V_1b, V_1c, mesial, ventral, and lateral terminal branches of the profundus nerve.



TEXT-FIG. 3.

Diagram of the development of the extrinsic eye muscles of Urodela to show the ontogenetic stage reached in Proteus anguinus. All diagrams show the muscles of the right side, with the anterior of the animal to the right of the page. A, 17-mm. Menopoma larva; B, adult Proteus anguinus; C, 18-mm. Menopoma larva. A and C after Edgeworth, B original. *VMP*, vertical muscle plate; other lettering as in Text-fig. 2.

optic nerve and the artery, below the M. levator mandibulae anterior, towards the eye. It broadens and divides, giving rise to the following muscles:

(1) M. rectus externus (*Mre*) runs backwards and upwards behind the optic nerve to its insertion on the postero-mesial face of the eyeball.

(2) M. rectus inferior (*Mri*) runs forwards, below the optic nerve and the choroidal branch of the ophthalmic artery, and upwards to its insertion, very close to that of the inferior oblique muscle, on the antero-ventral surface of the eye.

(3) M. rectus superior (*Mrs*) passes forwards and outwards above the optic nerve and the choroidal branch of the artery towards the dorsal surface of the eye. In two other specimens it is inserted on the sclera very close to the superior oblique, but in this specimen it fails to reach the eyeball and joins the superior oblique by means of a thin fibrous strand 10–15 μ in diameter, laterally to the eye.

(4) M. obliquus superior (*Mos*) arises from the parietal wall of the orbit just above the processus antorbitalis and passes dorsally to all branches of the deep ophthalmic nerve and to the ophthalmic artery. It does not reach the eyeball, but is joined to the superior rectus. In two other specimens a muscle with precisely similar relations has a normal insertion on the sclera and there can be no doubt about its identity in this case.

(5) M. obliquus inferior (*Moi*) is a well developed muscle (80–100 μ in diameter near its origin, where it is broadest). It originates from the parietal in common with the superior oblique. It passes outwards along the processus antorbitalis, ventrally to all branches of the deep ophthalmic except the ventral terminal, and to the ophthalmic artery, to be inserted on the antero-ventral surface of the sclera just in front of the inferior rectus.

The M. rectus internus is always absent, and so are the M. retractor and levator bulbi.

The only conditions which require special comment here are those of the superior oblique and rectus and their inferior counterparts. These vary; either pair may have a normal insertion on the eyeball (as do the inferior oblique and rectus already described) or either pair may fail to grow on to the eyeball and remain attached to each other, like the superior oblique and rectus muscles in the specimen illustrated. These peculiarities and the absence of the internal rectus, which is constant, are explicable when the embryology of the extrinsic eye muscles in Urodela is taken into account (see p. 41).

The naso-lachrymal duct (Fig. 1, Pl. 1) is usually absent in Proteus, but occurs in two of my specimens; it consists of a long, straight canal, opening anteriorly at the border of the external naris into the nasal canal and passing backwards, laterally to the olfactory epithelium, to end in two or three blind branches below the skin of the orbit. Of the three branches of the duct, one, the mesial, ends just above the antorbital process, and the two others pass over this structure, one on each side of the maxillary nerve; of them, the inner ends just anteriorly and the outer just posteriorly to the eye. The duct consists entirely of a simple cubical epithelium, without cilia, and closely resembles the embryonic duct at an early stage of development in Amphibia, as described by Born (1877), and the cells show no signs of degeneration. There is no sheath (propria) of any kind. The presence or absence of the duct has nothing to do with age. It was best developed in one of my largest animals (26·5 cm.) and absent in all smaller individuals except one.

According to Schlampp the skin above the eye is specially modified to form an organ which he called the accessory cornea of the epidermis ('accessorische Hornhaut der Epidermis'). I have examined sections of skin from most regions of the body of Proteus, including the head, trunk, and tail, and find that, apart from the absence of chromatophores, the epidermis is identical with that of Necturus (Dawson, 1920) and calls for no special description (Fig. 1, Pl. 1). Its most striking feature is the presence everywhere of numerous large cells of Leydig. These cells, whose function is not known, are characteristic of larval urodeles and ordinarily disappear at metamorphosis; their disappearance, in fact, is accepted by modern experimentalists as a reliable criterion of transformation (Reis, 1930, p. 497). Schlampp was impressed by those of

Proteus, and maintained (1) that they were especially numerous in the region of the eye, (2) that they were finely granular, highly refractive, and pellucid, and (3) that they resisted attempts at staining. He considered that they functionally replaced the cornea in that they formed a specially transparent and refractive area of skin admitting light to the eye. Kohl (1892) accepted his findings. I disagree with all his assertions. The Leydig cells are certainly numerous on the head, but not specially so near the eye; indeed, in sections of this region it is often possible to count more of them in other places (e.g. round the lips). They are coarsely granular and certainly cannot be regarded as pellucid, and they stain brilliantly with eosin, erythrosin, aniline blue, and light green. Schlampp's figure of the cells gives a clue to the origin of his mistake. In sections of my material fixed in corrosive sublimate the cytoplasm of the Leydig cells had contracted, leaving just such large empty spaces as those in Schlampp's figure of his 'accessory cornea'. I do not doubt that this error in Schlampp's otherwise careful account was due to faulty fixation.

III. Experimental Investigations.

1. Material and Methods.

Only one species of Proteus is now recognized, the divisions of the genus made by Fitzinger being regarded as invalid, even as varieties (Spandl, 1926). However, to ensure getting healthy animals and as much uniformity as possible, I went to Jugo-Slavia and collected animals for experimental purposes from one source, an unnamed cave near Kočevje in Slovenia. The native waters of Proteus are commonly at 9–12° C. though, occasionally, the temperature may be a little higher. Care was taken to keep the animals always below 15° C. They were kept for a time at Cambridge in a constant-temperature room at 10° C. and later in Manchester, where they were lodged in a thick-walled, darkened room at a temperature of 9–12° C. At its maximum, the stock consisted of seventy-three animals. They were fed on tubificid worms and weekly weighings were recorded at least three times before any experiments were begun, and again afterwards.

At first, standard light was obtained from a lantern projector with a 500-watt bulb and passed through an Ilford Daylight filter (no. 810). This was discarded later and direct lighting from ordinary frosted bulbs was used. Intensities were measured directly by means of an Avro-Light Meter, and the error involved was negligible in comparison with the differences between the experimental intensities. Different lamps do, of course, supply spectra which are not strictly comparable. To see if the error involved in neglecting this difference was large enough to affect the results, lights of different wattages were arranged to give the same intensity at a given level, and the reaction times of two animals recorded (Table I). When the figures obtained are compared with the body of results in Table IV, it is clear that the error is so small that, if it has any effect at all, it may be disregarded.

TABLE I. *The mean latent period of reaction in seconds of 2 animals to 8,600 candle metres from lights of different wattages. The times are averages of 25 readings each.*

	Mean latent periods of reaction.		
Animal No.	*100 W.*	*60 W.*	*40 W.*
8	26·5	27·7	29·2
14	24·3	27·2	25·9

To obtain a thin pencil of light, in testing the sensitivity of different parts of the body, a G.E.C. hand-torch with a 4·5 V. 3-cell battery and 3·5 V. 0·3 amp. bulb was used. The bulb chamber was blackened and a diaphragm with pin-hole mounted just in front of the bulb. In place of the ordinary glass front of the torch a metal face was screwed in to hold a cylindrical jacket in which a lens holder could slide to and fro in front of the pin-hole. This simple apparatus gave a bright spot light of any diameter from 3–6 mm., and had the advantage of being easily manipulated. With a little practice it was possible to hit any required spot on an animal, whether still or moving, by setting the torch by a safe light before switching on.

Two safe lights, to which animals were unresponsive, were used, one for general faint illumination of the room, and one for observing the position of an animal before an experiment. The former was a 100-watt frosted bulb, thickly painted red, and transmitting between 6,340 and 6,640 Å approximately. This produced no response from an animal at four feet; but, in practice, it was never allowed to shine directly on an experimental animal. For direct illumination a similar torch to that described above was used, but fitted with a Kodak Filter No. 44, mounted between frosted and plain glass plates and transmitting from 6,100 Å onwards. This produced no response at two feet.

The first part of this work was done at 10° C. in a constant temperature room, but at Manchester, although the severe winter of 1939–40 kept the temperature down, there was some variation; maximum and minimum readings for the experimental period were 12·7° and 9·0°. As the higher temperatures occurred at the end of the period, it was possible to test at 12·7° the reactions of animals already tested at the lowest temperatures. The results show that the slow changes experienced over eleven weeks do not affect the reaction times (Table II).

Table II. *Mean latent periods of reaction in seconds to 26,500 candle metres at the highest and lowest temperatures experienced during the experimental period. The times are averages of 50 readings each.*

Animal No.	*Mean latent periods of reaction at*	
	9–10·7° C.	*12–12·7° C.*
28	12·4	13·1
29	11·6	12·0
30	14·2	12·8

It was regularly part of all experimental procedure to introduce an animal into the experimental tank at least twenty-four hours before it was used. Once in the tank, it was left undisturbed. If it was needed in a particular part of the tank I always waited until it went there of its own accord. This necessarily caused great delay in the work, but from preliminary observations I came to consider it of the utmost importance to avoid handling the animals. It is clear from many papers that more hasty methods make it impossible to be sure that responses ascribed to the action of light have not been confused with those due to tactile stimuli.

2. Photosensitivity of Eyes and Skin.

It is evident from the condition of the eye that this organ is incapable of forming any image, but anatomical study shows no reason why it should not play a part in the general perception of light by the animal, such as that claimed by Dubois (op. cit.). His claims were tested by the following methods.

Light from the 500-watt lantern projector through the daylight filter was used throughout this series of observations. The projector was mounted on a turn-table, placed on a firm base, and could be revolved to aim at the door of any one of five boxes, arranged in an arc about the lamp. Each box was painted black inside and light proof. The upper part of the face looking towards the projector was a door, opening downwards and outwards. On the floor of the box rested a circular aquarium, 12·5 cm. in diameter, and containing water 3·5 cm. deep. Above the aquarium slanted a mirror in the roof of the box, so that, with the door open and the light on, the beam from the projector was reflected down into the aquarium and the animal's movements could be observed in the mirror, from behind the lantern. The door of the box was felted and could be opened quickly and noiselessly. The lamp switch and lantern were on one table, and the boxes on another, and there could have been no transmission of vibration when switching on. All experiments were performed in a constant-temperature room at 10° C. with a stone floor and black walls.

The procedure was very simple. With an animal in each box, the safe light was turned on, the door of the box was opened, the lantern and stop-watch switched on, the reaction observed in the mirror and the stop-watch switched off. Fifteen minutes were allowed between tests on any one animal. After a number of exploratory trials, a specific response (the 'turning response' described below) was noted, and used as a standard to mark the end of the latent period of reaction. The timing was assisted by the extreme sluggishness of the animal; when first observed as the light came on, it was almost always seen to be lying motionless in the bottom of the aquarium. In fact, in 3,325 tests made in this and other series of experiments, on only 1·35 per cent. of the occasions was an animal found to be moving at the beginning of an observation. In this minority of cases the experiment was cancelled. Thus, the latent period of reaction was that between illuminating a stationary animal and the beginning of its motor response to light.

This response was very regular and sharply defined. At the end of the latent period the animal twisted suddenly round and began to swim quickly in the opposite direction to that of its original posture. The form of the response is illustrated in Text-fig. 5, though under different conditions from those described here. As it will be necessary to describe other responses of Proteus to light, I shall refer to the simple reaction outlined above as the turning response. It was elicited not only by light, but by sudden and violent vibration (heavy thumps on the table, jogging the aquarium) and by fairly violent tactile stimuli (pinching the tail, striking the back with a glass rod), but it could not be obtained by slight vibration or more gentle tactile impressions, though both of these caused movement. There can be no doubt, then, that the regular occurrence of the turning response was due in these experiments to the light, all other more violent stimuli being certainly excluded. Its consistent form made it easy to distinguish the true light response from casual but very rare movements, such as a slight raising of the head or displacement of a limb.

Five animals were selected as the most consistent in their reactivity, for 'blinding'. For the reasons given below, an attempt to use Dubois' method of blinding was abandoned as unsatisfactory, and other means were tried. Finally, the animals were induced to wear tiny spectacles fitting closely over the eyes and strapped on by means of cotton laces tied under the chin. There were two kinds of spectacles. In one, used for controls, the 'lenses' were of perforated paper which passed the light, but acted as tactile irritants in the same way as the dark spectacles, which had 'lenses' of thick dark leather. No readings were taken until the animals had worn their spectacles for two days, by which time they appeared to be perfectly normal in their behaviour.

Twenty readings were taken from each animal, first with the control, and then with the dark spectacles. The latter were then removed, and the animals again tested in normal conditions. The results are shown in Table III. Those from all animals are quite consistent enough to be the basis of a fair comparison. This seemed obvious from inspection, but to establish the fact beyond doubt, the standard errors of difference for control and experimental results were calculated; this showed that the slight differences between the average reaction times in the second and third columns are quite without significance in every case. It is concluded that blinding makes no difference to the reaction times of the animals. On this evidence alone it is of course conceivable that the eyes are still functional in some way; the retina contains normal, though embryonic, visual elements as well as degenerate ones, but if any function is to be allowed to these cells, it has a maximum effect so much smaller than the total effect produced by the skin receptors that its subtraction makes no detectable difference to the latent period of reaction to illumination; the doubling of the reaction time reported by Dubois is out of the question.

Table III. *The latent periods of reaction to light of constant intensity. The experimental animals wore opaque and the controls transparent spectacles; normals wore none. Each figure is the mean in seconds of twenty readings made in tenths of seconds.*

Animal No.	*Mean latent periods of reaction.*			*Mean Differences.*	*Standard Errors.*
	Normal.	*Control.*	*Experimental.*		
8	15	16	13	3	2·07
9	17	18	17	1	2·29
13	16·5	—	16	—	—
14	24	20	25	5	3·57
15	14	17	15	2	2·91

As my results so specifically contradict those of Dubois, it may be well to offer some explanation of his findings. He performed very few experiments and expressed his results in averages only. He reported nothing of the condition of his animals or the way in which they were kept. Proteus varies a good deal in its reaction times and it is essential to employ only consistent, healthy animals for experiments and to make a large number of readings before extracting a reliable mean. But probably Dubois' most serious source of error was in his method of 'blinding'; he covered the eyes of his animals with a mixture of gelatine and lampblack. I attempted this method and found it quite unreliable. The lampblack never stayed on for more than half an hour, so presumably Dubois' experiments were performed very shortly after handling the animals. Proteus is exceedingly sensitive to handling, and the violent stimulation involved in holding the animal and the irritation caused by the method were such that one animal of mine did not recover its normal sensitivity to light for fourteen days after the operation. Before recovery was complete it was always restless when examined in the light, and made occasional movements which, in the absence of any definite criterion such as the turning response (which Dubois apparently failed to notice) might have been taken for reactions to light. I consider it to be beyond doubt that these early results were based on thoroughly abnormal and unreliable animals.

3. The Photoreceptive Regions of the Body.

Dubois stated that the head and tail of Proteus were more sensitive to light than the rest of the body; differences in the sensitivity of the head and tail skin of a few other amphibians, e.g. Necturus and Cryptobranchus alleganiensis, have been reported by Reese (1906), Eycleshymer (1908), Pearse (1910), and Sayle (1916).

To investigate this point in Proteus the spotlight torch was used, the animal being touched with a 3 mm. circle of light at points on the dorsal, lateral, and ventral sides in two bands round the face, three round the body, and two round the tail. There is no need to treat each of these areas separately; all points on the head and cheeks can be taken together, and so can all those on the back and sides of the body and tail. The ventral surface of the animal is probably insensitive to light.

(1) Dorsal and lateral surfaces of the head.

Illumination produced the usual turning response. In twenty-eight out of thirty-six trials the reaction was complete and in the rest the movements could always be interpreted as the opening phases of the response. The latent period of the reaction was roughly of the same order as that obtained by illuminating whole animals—the average of thirty-six readings was 6·7 seconds.

The minimum intensity required to produce the above response was found by more accurate means than were possible with the hand-torch, but here it must be stated that as the intensity of the beam was diminished, the turning response was gradually replaced by the tentative movements described in the next paragraph. Such movements, when made by the head, are, of course, very difficult to classify; obviously a slight movement of the head to one side might, if it had developed, have been recognized as the opening phase of the turning response, but it may equally well be regarded as a special case of the general adjusting movements now to be described.

(2) Dorsal and lateral surfaces of the body and tail.

In twenty-five out of twenty-six trials a reaction quite different from the turning response was obtained. In these cases some slight movement of adjustment—a flick of the tail or shift of the body to one side—took the exposed spot out of the light and the movement stopped. Repeated teasing by following the animal with the light evoked a series of such tentative movements, leading to slow creeping or swimming, but not to the vigorous and decisive turning response. The latter was, however, given on a single exceptional occasion. The reaction time (average of twenty-six trials) was 2·6 seconds—much shorter than that required for the organization of the turning response.

4. The Effects of Different Intensities.

To study the relation between the latent period of reaction and the intensity of the stimulus, the animals were placed in the small glass dishes used in the preceding series of experiments. Each dish rested on the floor of a blackened compartment, 30 cm. square and 40 cm. deep and open at the top. There were five such compartments, each of which could be illuminated by the same Terry Anglepoise Lamp, which could be placed so that the animal in one compartment was lighted without affecting the others. The times were again taken from the moment that the light came on until the first movement of the turning response; casual movements were disregarded and if an animal was found to be moving at the beginning of any experiment it was cancelled.

The results are recorded in Table IV and graphed in Text-fig. 4. The animals mostly behaved consistently, except for some expected deviation. A few (Nos. 31, 36, 38, 45) fell outside the general trend and appeared to be set for a slower speed of reaction; they are segregated at the bottom of Table IV. The reaction times were more variable at low than at high intensities and the values of the standard errors ($\pm s_{\bar{d}}$) and mean/standard error (t) for the differences between the first and second columns are given in the last column of the table. As these were found in all cases to be highly significant, no statistical tests were applied to the more consistent figures on which the mean reaction times at higher intensities were based.

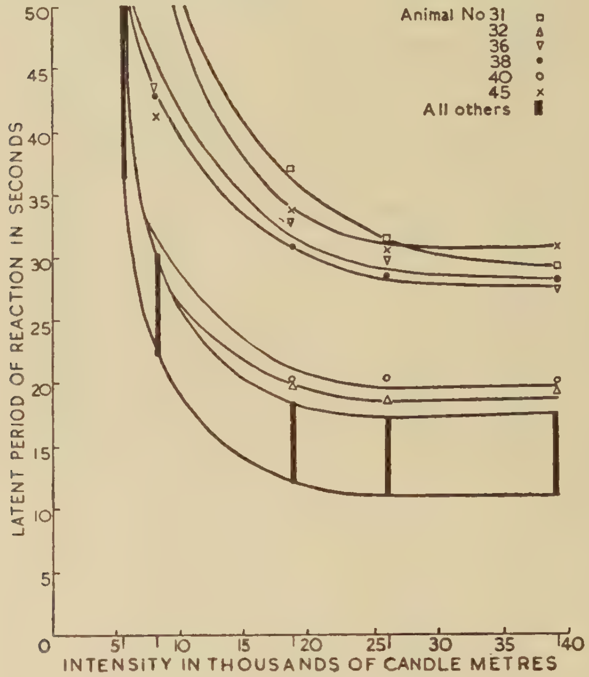
For all animals there is a rapid decrease in the latent period of reaction between 5,000 and 10,000 candle metres, and then a falling off up to 25,000 candle metres. Increases in the quantity of the stimulus are ineffective beyond this point, if not before. The more powerful stimuli produce more regular results, in range, in that more animals approximate to the same reaction times, and in that the response itself is more violent and clear cut. At lower intensities the reaction times of different animals spread over a wider range. The weakest stimulus used is just enough to produce repeatedly, in the majority of cases, a performance of the turning response, though this may be slow, and, rarely, incomplete. Evidently 3,500 candle metres is near the threshold needed for this reaction.

Table IV. *The mean latent periods of reaction of Proteus to light of different intensities. The times are the means in seconds of fifty readings for animals 28–37 and of twenty-five readings for 38–45. Nos. 31, 36, 38, and 45 are segregated as abnormally slow (see text).*

Animal No.	*Intensity in Candle Metres.*					*For columns 1 and 2.*	
	3,500.	*8,600.*	*19,200.*	*26,800.*	*39,550.*	$\pm s_{\bar{d}}$	$\pm t$
28	42·5	27·2	14·1	12·4	12·5	4·1	3·6
29	37·4	25·9	12·3	11·6	10·5	3·1	3·2
30	42·3	29·2	16·9	14·2	13·1	3·5	3·6
32	45·2	26·1	20·1	18·9	19·5	2·5	10·5
33	41·4	27·1	15·4	11·9	11·8	2·3	6·0
34	48·5	25·9	15·3	14·8	15·6	2·3	9·8
35	46·5	30·4	15·7	13·8	13·2	2·1	7·5
37	46·5	29·9	15·5	15·3	15·5	2·6	6·4
39	44·7	23·3	17·4	16·2	15·9	2·4	8·8
40	54·2	31·7	20·4	20·4	20·2	3·5	6·6
41	42·9	27·8	17·2	17·5	16·8	2·7	5·5
42	49·5	27·4	17·9	15·9	16·2	4·0	5·5
43	44·4	25·2	13·4	12·8	12·1	3·4	5·6
44	50·3	30·3	16·5	16·9	17·2	3·7	5·1
Mean Times	45·4	27·7	16·4	15·2	15·0		
31	—	107·7	37·1	31·2	29·2		
36	—	43·1	32·9	29·8	27·5		
38	57·6	42·9	30·9	28·5	27·6		
45	—	41·4	33·9	30·5	30·6		

The behaviour of the four slow animals mentioned above was different. At the lowest intensity so few turning responses were obtained, though the animals showed signs of disturbance, that the figures were discarded as unreliable for three animals. 3,500 candle metres was below the dependable threshold for them, and there were a few failures at 8,600 candle metres. This, and the fact that all reactions of these animals were slower throughout the range of intensities suggest that the readings are not experimental errors, but that these four specimens were in fact less responsive, reacting more slowly and only to higher intensities.

It must be noted that the threshold here given for the turning response is only approximate, and cannot be taken as a clear-cut boundary between stimulation quantities producing two different types of behaviour. It sometimes happened that stimuli well below 3,500 candle metres produced the turning response, but on repetition of the weak stimulus at 15-minute intervals this response quickly disappeared and was replaced by the slight avoiding movements associated with tail/body skin



TEXT-FIG. 4.

Graph to show the relation between the latent period of reaction of the turning response and the intensity of stimulation.

stimulation. Above the threshold the turning response could always be provoked at 15-minute intervals with only a very small number of failures.

5. Photophobotaxis.

Theories of orientation to light are based almost exclusively on the behaviour of invertebrates, in which the responses are relatively simple and regular and often respond to experimental analysis. But there is some evidence that the underlying capacity for such simple reactions still exists in vertebrates, though masked by the more precise and flexible reactions facilitated by the complex brain and eye. Crozier and Pincus (1926, 1927) have reported in young rats, before they open their eyes, a type of reaction (phototropotaxis) common among invertebrates; as soon as they open their eyes, however, this behaviour is replaced by straightforward movement towards the goal that they can now see. But there is no evidence that the simple orientating responses are ever lost in adult animals and it seemed that the case of Proteus might repay investigation.

During recent years a very elaborate nomenclature has been evolved to describe the reactions of animals to light (see especially the works of Loeb, 1918; Rose, 1929; Fraenkel, 1931; Fraenkel and Gunn, 1940), and to avoid confusion it seems desirable to state the sense in which the term photophobotaxis is used here. The characteristic features of this type of orientation are taken to be: (1) On encountering an unfavourable intensity of light the organism is arrested in its course and changes the direction of its movement (shock reaction): (2) the change in direction is not constantly related to the source of light, but is haphazard, and the component of the stimulus evoking the shock reaction is intensity, not direction: (3) the repeated shock reactions restrain the organism from entering unfavourable fields and trap it in favourable ones. The type of movement seen is often called 'trial and error', and the classic example of phobotaxis is provided by the reactions of Paramecium to various stimuli.

The following investigations were made to discover if the term as defined above could be applied to behaviour of Proteus based on the turning response.

(1) The turning response as obtained by sudden illumination of the whole animal has already been described. The cases in which the animal was found to be moving at the beginning of an experiment, so far ignored, must now be considered. It was observed that the movements in progress at the moment of illumination were never continued into the turning response; the first effect of light was to bring all movement to an abrupt stop; then, after a pause (latent period), the turning response was given just as if the animal had been still from the start of the experiment. A typical protocol was as follows:

Seconds

0·0 Light on, 19,200 candle metres. Animal slowly creeping over bottom of tank.

1·3 Sudden, complete cessation of movement. Animal held rigidly in creeping position. Body slightly flexed.

15·8 Sudden and rapid turning response, animal swimming off rapidly at an angle of about 140° to its original direction.

The total effect, seen again and again in these and the following experiments, was to prevent further exploration in the original direction and precipitate movement along a new line.

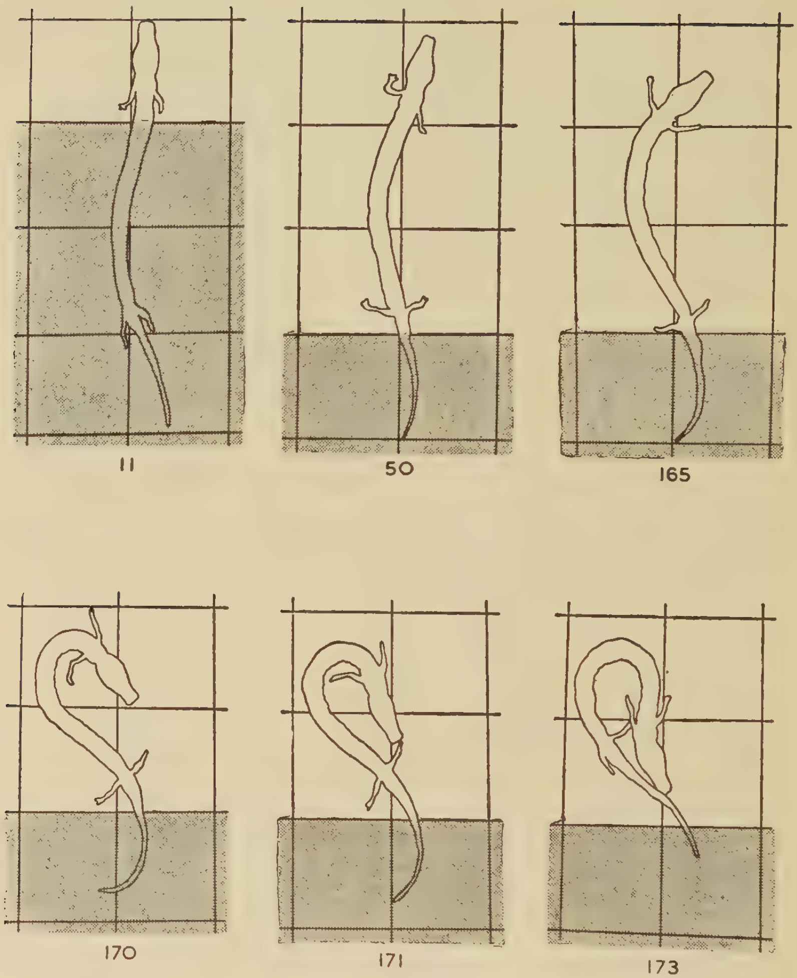
The reaction was more easily studied in tanks shaded so that one half was dark or dim and the other half fully illuminated. One half of the tank was roofed with black paper and a black curtain hung down across the half-way line almost to the surface of the water. This arrangement gave a clear-cut boundary between the dim or dark and the light halves. To obtain dim and light fields the thick black paper was replaced with thin white, or the tank was illuminated by a projector, the lens being shaded to cast a shadow on one half of the tank while fully illuminating the other. One animal at a time was tested, after being left in the tank for a day in total darkness. It could always be driven into the dark or dim half by illuminating the other half 15 minutes before an experiment. Movement out of the dark was elicited by gently tapping the wall of the tank.

Taking into account only those experiments in which an animal passed through the whole cycle of its response without touching the sides of the tank, there were thirty-five trials. Inhibition occurred from 0·3 to 2·1 seconds after the tip of the snout entered the light, and the animal was usually arrested with about half its body in the light. A cinematograph film was made of shock reactions at the dim/light boundary and projections of sections of it traced. Some of these, all from the same reaction, are shown in Text-fig. 5. The figure beneath each tracing is the number in the series of the exposure, counting as zero that taken as the tip of the animal's snout crossed the boundary; there were ten exposures per second, so that the times between the different stages of the reaction can easily be calculated from the figure.

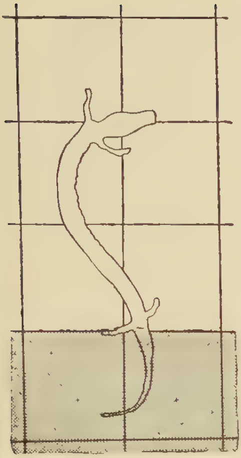
The first picture shows the animal 1·1 seconds after the boundary had been reached; the limbs were still in the normal swimming position for Proteus. They were brought forward to check swimming 2 seconds after 0·0, but during the next few seconds slight adjustments in the position of one hind limb were made, apparently to maintain the creature's balance. From no. 50 to no. 165, a latent period of 11·5 seconds, there was complete immobility and then the turning response, figured in the last ten pictures, and taking 1·5 seconds, was given. A turn of about 180°, as figured, was the most common, and almost always the turn exceeded 90°, so that the animal was sent back into the dark.

(2) A further distinctive feature of photophobotaxis is that the path taken by the animal is not constantly related to the position of the source of the stimulus; the path taken after the shock reaction varies. It has already been mentioned that, with light from a fixed source, the angle of turn varied, though usually between 90° and 180°; further it might be to the right or the left. Confirmatory evidence was obtained as follows.

A rectangular glass tank was divided into dark and light halves and lined with black paper throughout, except for one or the other side of the light half of the tank. Through the unlined wall light from a small Leitz Projector was admitted, to pass transversely across the path of an animal entering from



TEXT-FIG. 5. The turning response



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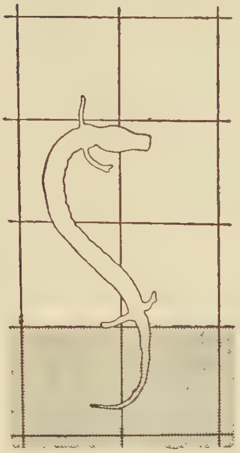

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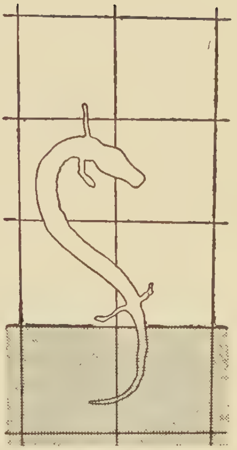

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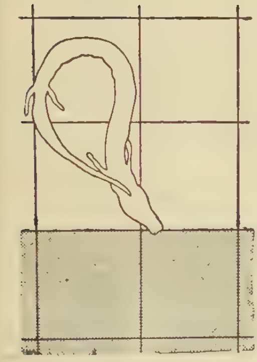

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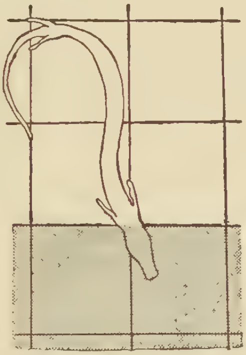

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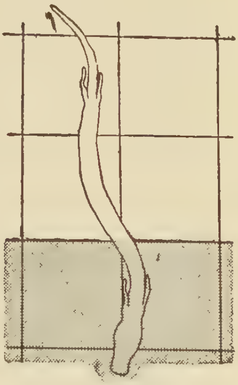


180

of Proteus anguinus.

the dark field. Animals were separately tested with light coming from the right and from the left and the results are shown in Table V; it is apparent that there is no relation between the source of light and the direction of turn.

TABLE V. *Similarity of turning responses obtained by illuminating animals from either side.*

Details of response.	*Illumination from*	
	Left.	*Right.*
Turning response to left	14	13
,, ,, right	16	17
Angle of turn about 180°	18	19
,, ,, 180°–90°	12	10
,, ,, about 90°	—	1

It is also evident that the response under the conditions so far described was to a change in the intensity of illumination, not to direction. The behaviour of animals at the boundary between directional and non-directional light suggests that this is generally so. Such a boundary was set up as follows. Non-directional, diffuse light was obtained from four electric light bulbs mounted behind a sheet of frosted glass. The source of directional light was a lantern projector used from the side. The tank was divided by a black cardboard curtain, extended upwards and outwards to separate the light sources. The intensity on both sides could be varied by adjusting the positions of the lights. The floor and walls of the tank (except the half of the wall facing the projector) were lined with dull black paper. Five animals were separately tested five times each under all the following conditions.

The lights were arranged to supply equal intensities in both fields of approximately 100 in the first and 15,000 candle metres in the second series of trials. In not a single case was any shock reaction observed in passing from one to the other field, though, of course, the onset of the higher stimulus caused a shock reaction, whether the animal received it in the directional or non-directional field.

The effect of unequal illumination was tried by raising the intensity of each half in turn while keeping the other half at 100 candle metres. It was found that—presumably owing to the operation of the Weber-Fechner Effect—no shock reaction was evoked until the brighter side reached an intensity of 34,000–40,000 candle metres. Below this level the result was the same aswith equal in tensities; above it, in every one of the fifty trials at 40,000 candle metres or more, the shock reaction was given whether the passage was from directional to non-directional light or vice versa.

It is not claimed that these simple experiments rule out all possible effects of qualitative change in the components of the light stimulus; it may be that directional gradients play some part in guiding animals over large areas, as they may do when the stimulus is very weak (see below). The evidence submitted above is meant only to show that shock reactions follow the recognition of sudden and considerable changes of intensities, which accords with the interpretation of the reaction as photophobotactic.

(3) The number of occasions on which the turning response was made through an angle of 90° or less and then failed to drive the animal back into the dark was always very small (see Table V). Other means of trapping Proteus in the dark exist and are described below, but here it is sufficient to note that, at intensities above the threshold for the shock reaction, this response is most effective in fulfilling the usual function of photophobotaxis.

6. Behaviour below the Threshold for Photophobotaxis: Photokinesis.

The threshold for shock reactions is fairly high and rises rapidly (to 34,000–40,000 candle metres with an initial stimulus of 100 candle metres), and there are many dim/ or dark/light boundaries at which no shock reaction takes place. Nevertheless, if animals are left long enough in a tank under conditions which preclude photophobotaxis, they do find their way to the dim or dark fields and stay there. The results are so similar to those obtained by Young (1935) with lampreys that his description of their behaviour might serve admirably for

Proteus. On illumination the animals which happen to be in the light half of the tank begin to move, swimming in and out of the light, disturbing those in the dark, which also swim about. The movement is haphazard and disorderly; animals settle down occasionally for some seconds in the light, but sooner or later begin to move again. There comes a time when all the animals are assembled in the dim or dark area. Most careful observation revealed no trace of orientated migrations; such activity in which light excites organisms to random movements which carry them by chance into the dark has been called photokinesis. This was Young's interpretation of the behaviour of his lampreys and, at first sight, there seems to be no reason for withholding it in the case of Proteus. We should then conclude that Proteus finds its way from light to dark or dim places by means of two different types of behaviour, photophobotaxis at high, and photokinesis at low, intensities.

The following considerations suggest that this simple explanation may be too easily accepted and requires further examination. (1) When the head of Proteus alone is illuminated at decreasing intensities the shock reaction gradually gives way to the irregular and tentative movements described on page 25. (2) In one known case at least, that of Dendrocoelum lacteum, the simple explanation suggested above for Proteus has been shown to be erroneous (Ullyott, 1936); these planarians moved about apparently at random in the dark, but showed shock reactions on encountering light above a certain intensity; Ullyott was able to show, by analysing the tracks left by the animals, that these two apparently distinct types of behaviour were really only extreme manifestations of a single basic tendency and connected by intermediate sorts of reactions. (3) The assertion that the movement of Proteus —and, presumably, that of lamprey larvae—is, under certain conditions, photokinetic depends on a subjective impression of randomness.

It was not possible to record the tracks of Proteus for analysis and the method designed to test the apparent randomness of its movement was based on a corollary of photokinesis.

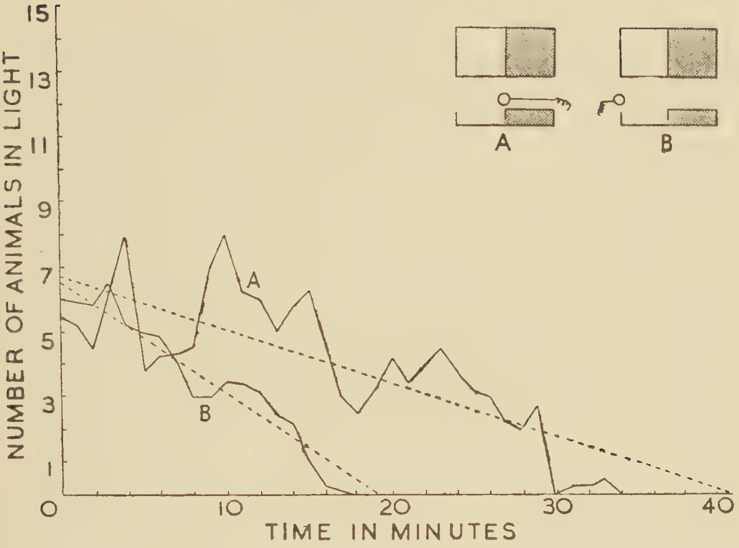
Since in any field illuminated at an intensity provoking photokinesis all movements are at random, it follows that the field of stimulation is uniform throughout in effect; for, if slight variations in any factor (e.g. intensity) were translated into differential activity affecting orientation, the movements would not be random. Within the limits for photokinesis all light produces the same response, and small variations in the fields will not produce any consistent trend altering the manner of migration from light to dark. The object of the following experiment was to record migrations from light to dark in two types of apparently photokinetic fields differing slightly in the distribution of intensity at the light end, and to compare results. If no consistent differences were found, all movements would be truly random and the conditions for photokinesis fulfilled; if, on the other hand, significant trends were established, they would indicate the existence of determining factors, even if subjective observation could merely disclose apparently random activities.

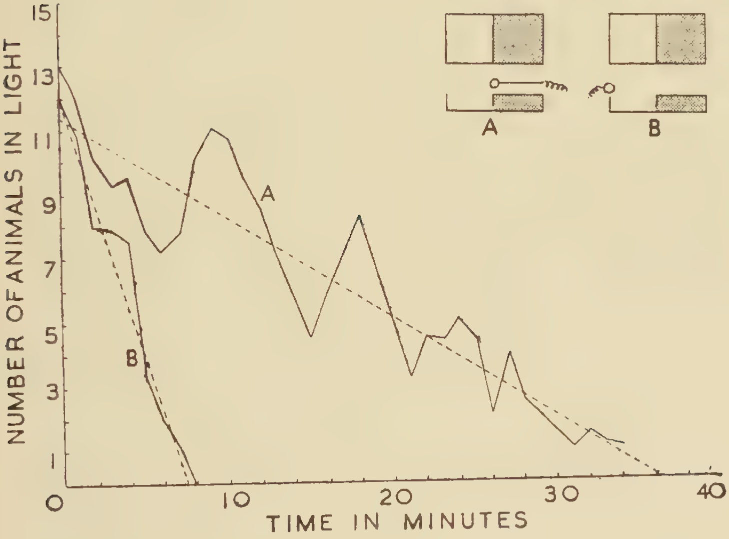
A large stone tank (88 × 52 × 11 cm.) contained water 2·5 cm. deep in a constant-temperature room at 10° C. One half was roofed with a plywood cover and a thick cardboard curtain, almost touching the surface of the water, bisected the tank transversely. The other half was covered with two glass plates. Weak illumination from a 25-watt frosted bulb could be obtained at two points, at *A*, above the centre of the tank, and at *B*, above the midpoint of the side opposite the curtain, at the far end of the 'light' half of the tank (see diagrams on Text-fig. 6). With the light at *A* there was an intensity gradient diminishing from the dark half; with the light at *B* it led towards the dark half. Fifteen animals were used, and these were tested, singly and together, with the light first in one and then in the other of the two positions, so as to ensure that no shock reactions occurred (illumination below the threshold for photophobotaxis) and that movements were in both cases apparently at random (conditions evoked apparent photokinesis).

The procedure was as follows. Fifteen animals were left in the tank overnight. Next day, the light at *A* was turned on and a stop-watch started simultaneously. The initial number of animals in the light half was noted and all exits and entrances of animals recorded with their times. In addition, quick sketches (on prepared plans of the tank) were made as often as possible, showing the positions of the animals where they paused. It was found possible to record up to eight entries and exits (the maximum performed) and two sketches per minute. A check was made by an independent observer, noting the number of animals in the light every 15 seconds. An experiment ended when all the animals reached the dark and stayed there for 5 minutes. Subsequent observations at 5-minute intervals showed that, once this state was achieved, animals, with very few exceptions, usually stayed in the dark for some hours, that is, responses to light in the absence of photophobotaxis were certainly efficient in driving animals into and keeping them in the dark. No experiment was continued after an hour, as there was evidence that, after that, either adaptation or exhaustion interfered with the light response; but, in practice, this arbitrary curtailment was unimportant: seven times out of twenty, at the end of an hour, a single unresponsive animal was left in the light, while the other fourteen were in the dark, and once three animals remained, unmoved, in the light. Nine experiments with the light at *A*, and ten at *B* were made; and for half of each class the light and dark halves of the tank were reversed for the sake of control. After preliminary trial experiments the temperature before and after an hour's exposure to light was taken in the four corners of the tank and just below the light. In one case a general rise of 0·5° was noted, in two others, none. The glass prevented too much temperature change below the lamp and the movement of the animals stirred the water. Most experiments lasted considerably less than one hour.

The results of each experiment were separately graphed. From the record of entries and exits the actual number of animals present at the end of each 15 seconds was written out and the average number for each minute found. The figures were plotted against time for each experiment and the regression lines calculated by the method of least squares. From these data the best lines were drawn (broken lines in Text-fig. 6).

Two pairs of graphs are reproduced in this figure. In each


NUMBER OF ANIMALS IN LIGHT
15
13
11
9
7
5
3
1
0
10
20
30
40
TIME IN MINUTES
A
B
A
B


NUMBER OF ANIMALS IN LIGHT
15
13
11
9
7
5
3
1
0
10
20
30
40
TIME IN MINUTES
A
B
A
B

TEXT-FIG. 6.

pair the longer graph shows the effect with the light at *A*, the shorter, steeper graph at *B*. The graphs were chosen because the members of each pair were easily comparable at a glance, owing to the fact that the animals present at the beginnings of the experiments were nearly equal; this method of selection has nothing to do with the purpose of the experiment and, in fact, both *A* and *B* graphs are representative of their groups.

Both figures at once suggest that, under otherwise comparable conditions, with the diminishing gradient leading to the dark, the animals escape more quickly than when the gradient increases towards the dark. This was found to be generally so. With the light at *A* the times taken for total evacuation were 34, 32, 33, 19, 52, 41, 34, 19, and 38 minutes; with the light at *B* they were 21, 25, 12, 10, 19, 21, 14, 26, 18, and 8 minutes. The averages, 34 and 18 minutes, show that it takes twice as long to evacuate up the gradient as down it.

I conclude, therefore, that, although all movements were apparently at random, the alteration in the position of the light source did have a constant effect on the way in which animals escaped to darkness. The haphazard movement which characterizes photokinesis could not adequately explain this fact.

IV. Discussion.

1. Anatomical: The Influence of Neoteny.

Schlampp was fully aware that the condition of the eye of Proteus was the result of arrested development at the secondary optic cup stage, followed by degeneration. It seems likely that he overemphasized the latter factor. One of the most striking features of my material was the picture it presented not so much of a degenerate as of an embryonic structure. The histological characters of the sclera, choroid, and pigmented epithelium were those of healthy tissues; the retina was that of a typical amphibian embryo and well fixed material which had not been exposed to heat in the embedding oven (i.e. celloidin embedded material) contained many normal, though embryonic visual cells, in spite of the fact that they must have been inactive after their development stopped. Even the structure of the naso-lachrymal duct was precisely that described by Born in amphibian embryos. The most abnormal feature—and the one which conveyed an immediate impression of degeneration—was the cellular mass proliferated from the border of the optic cup. This and the condition of the extrinsic eye muscles call for special consideration.

In regard to the latter there is no complete account, so far as I am aware, of the development of the extrinsic eye muscles of a urodele, but Edgeworth (1928) has figured and described three stages in Menopoma, and stated that conditions in Hynobius and Necturus are substantially similar. According to him, in a 17 mm. Menopoma larva, there is a vertical muscle plate just behind the eye; anterior dorsal and ventral projections from it represent the primordia of the superior and inferior oblique muscles; the external rectus is a small bud at the lower end of the plate (Text-fig. 3, A). In the next stage (18 mm.) these three elements are separate and the vertical plate has differentiated into the anlagen of the superior and inferior rectus (C). The internal rectus develops later as an outgrowth from the upper end of the inferior rectus. The relevant point here is that there exists a stage when (*a*) the superior oblique and superior rectus are joined at their ends of insertion, (*b*) the inferior oblique and inferior rectus (lower portion of the vertical plate) are joined at their ends of insertion, (*c*) all the rectus muscles whose primordia are distinguishable at this stage (superior, inferior, external) are joined at their ends of origin, and (*d*) the internal rectus cannot be identified. Immediately after this stage the superior and inferior elements must separate into their oblique and rectus components. Which set separates first or whether one regularly does so before another is not apparent, but since these events take place between the 17- and 18-mm. stages, it is reasonable to suppose that their order of occurrence can hardly be of much account.

These embryonic conditions are just those occurring in the adult Proteus (Text-figs. 2 and 3). Variations in the superior and inferior muscles all approximate very closely to the conditions which must exist between the two known stages figured by Edgeworth; the internal rectus, the last to appear in urodeles, is always absent in Proteus; lastly, the structure of the muscles is histologically indistinguishable from that of other voluntary muscles and shows no signs of deterioration. It can hardly be supposed that so precise a correspondence between the conditions in the adult Proteus and in the embryos of other urodeles is the result of irregular and haphazard degeneration following loss of function.

In the organs so far considered there has been at least a partial loss of function, the effects of which (e.g. loss of lens) accompany those of neoteny. Fortunately, a survey of even so limited a region as the orbit reveals an example of straightforward neotenous development that can scarcely be the result of functional change. This is the form of the chondrocranial wall of the orbit.

The structure and development of the orbit in Urodela is well known and has been summarized and figured by de Beer (1937). At an early stage the trabeculae cranii are simple cartilaginous rods lying on each side of the brain; dorsally to them the optic nerve and ophthalmic artery pass outwards to the eye. Later, the side wall of the orbit appears as an orbital cartilage, which develops independently of the trabecula of its side (Necturus, Triton) or in continuity with it (Amblystoma). In either case the orbital cartilage becomes directly connected with the trabecula by vertical struts, the pila prooptica and pila metoptica, which enclose the optic nerve in front and behind in an optic foramen, whose roof and floor are formed by the orbital cartilage (now sometimes called the crista trabeculae) and the trabecula cranii respectively. The ophthalmic artery emerges behind the pila metoptica in company with the oculomotor nerve. Finally, in the great majority of Urodela, an ossification beginning in the pila prooptica spreads backwards towards the optic foramen to form the orbitosphenoid bone. Thus the definitive wall of the orbit is typically formed from the chondrocranium and its bony derivative.

As Text-fig. 1 shows, the condition in the adult Proteus is that of the earliest embryonic stage described above. The trabecula in this region remains throughout life a simple cartilaginous rod with the optic nerve and ophthalmic artery passing dorsally to it. The stage is never reached at which the orbital cartilage and orbitosphenoid develop, and these elements are functionally replaced by the orbital wing of the parietal bone. It is of interest to note that a stage intermediate between the early one represented by Proteus and the typical orbital structure in a urodele is found in the adult Necturus, the cranial development of which has been studied by Platt (1897), Winslow (1898), and Shumway and Webb (1932). Here an orbital cartilage is formed and joined to the trabecula in the usual way, so that nerve foramina are enclosed; but the crista trabeculae is reduced, the orbitosphenoid never ossifies and, as in Proteus, most of the wall of the orbit in the adult is formed by a large process of the parietal and the contribution from the chondrocranium is insignificant (Huxley, 1874). The chondrocranial component of the orbit in Proteidae is neotenous, but development is arrested at an earlier stage in Proteus than in Necturus.

There remains for consideration the mass of cells proliferated from the border of the iris and occupying, in most of the eyes studied, the place of the lens; when the lens is retained this proliferation is inhibited. From the studies of the last fifty years on the activity of the amphibian iris following loss of the lens, the following facts emerge. If the lens is extirpated, the cells of the dorsal border of the iris divide and their products differentiate into a lens (Colucci, 1890); even if so much tissue is removed that only the iris and pigmented epithelium are left, the eye as a whole is reconstituted (Wachs, 1920). The cause of the proliferation is not the mechanical stimulus provided by the removal of the lens through the pupil and the location of the activity in the dorsal border of the lens has nothing to do with gravity (Wolff, 1895, 1901); indeed the potency of the iris to proliferate extends all round its border (Sato, 1933), though, normally, it is the dorsal region which is active and, once its dominance is established, the potencies of the rest of the iris are inhibited. Spemann (1905) showed that a condition for what came to be called Wolffian regeneration from the iris is that the optic cup and epidermis must be separated by a layer of connective tissue, as in Proteus (Fig. 1, Pl. 1); when cup and epidermis are in contact, a lens is induced from the latter in the well-known way and the activity of the iris is inhibited. It is important to note that (1) if the dorsal border of the iris did not establish its inhibitory dominance, and proliferation took place all round the iris border, Wolffian regeneration of a lens would be impossible, and (2) the capacity of the iris to proliferate is a fundamental property of its margin, independent of the determination of its products to form a lens: ventral segments of the iris implanted into lensless eyes proliferated and lost their pigment, but did not differentiate (Sato, 1933). It seems clear from all this that the condition which in Proteus appears at first sight to be an abnormality due to some degenerative process not understood is in fact an expected result of the loss of the lens, except that no dominant region is established and proliferation from the whole iris margin takes place in the absence of the organizing influences supposed to reside in such a region. With this fact must be associated the failure to regenerate a lens.

It is contended here that the above facts provide a rational explanation of the condition of the eye of Proteus without recourse to such vague agencies as 'degenerative change following loss of function'. The only fact which cannot be related to known processes of growth or development is the loss of the lens itself; granted this, and the fact of neoteny, and the structure of the eye follows.

The general importance of neoteny in evolution has been argued by de Beer (1930). The interest of the present example lies in that degeneration of eye structure, loss of pigmentation and so forth among cave animals have been so often used to illustrate inconclusive arguments about the effect of loss of function on structure. Even now there is fundamental disagreement on these questions, so that Chapman (1931), having in mind the 'sceptical attitude of genetical research' towards the inheritance of acquired characters, writes of blind troglodytes that 'biologists of to-day find themselves in an embarrassing situation when considering such cases'. I submit that in this case, when a precise picture of the developmental changes which lie behind the ambiguous term 'degeneration' is considered in detail, a reasonable explanation can be suggested.

Of all Urodela the Proteidae are probably the most far gone in neoteny, as is shown by the incompetance of their tissues to respond to metamorphic agents and in Proteus the neotenous influence affects even small details of the form of the chondrocranium. Presumably the eye would be preserved in a functional state so long as it was of any use to the animal, but the adoption of the cavicolous habit would permit the existing neotenous tendency to extend to that organ. The agency for reduction of the eye was already present when Proteus led an epigean life and waited only for the opportunity to express itself. The question of the inheritance of acquired characters does not arise to disturb the simplicity of the argument; the blindness of Proteus is due primarily to neoteny and it is a character not determined, but permitted, by the nature of the environment.

2. The Light Reactions of Proteus in Nature.

Photosensitivity has been studied in other amphibians: in Urodela by Banta and McAtee (1906), Reese (1906), Smith (1907), Eycleshymer (1908), Pearse (1910), Franz (1913), Laurens (1914), Sayle (1916), Uhlenhuth (1921), and Cole (1922), and in Anura by Korányi (1892), Parker (1903), Torelle (1903), Pearse (1910), Laurens (1911, 1914), Franz (1913), Riley (1913), Cole and Dean (1917), and Kramer (1933). The number of species investigated has been small and they represent, especially among the Anura, only a few families (Pipidae, Bufonidae, Ranidae). The methods used were seldom free from objection; animals were handled just before or during experiments; detailed descriptions of the responses were seldom given; information about the numbers of animals and the intensities of illumination used is lacking; reaction times were omitted altogether or else so few readings were quoted that the results cannot be accepted. But while precise and detailed knowledge about the reactions of these animals to light is not available, certain consistent tendencies in their behaviour emerge. On the whole, frogs and toads move towards the light[1] (Bufo americanus, Bufo fowleri, Rana clamata, Rana pipiens, Rana sylvatica, Rana virescens, and various frog tadpoles), while urodeles move away from the light (Cryptobranchus alleganiensis, Amblystoma, Plethodon cinereus erythronotus, larvae of Spelerpes maculicandus, and Necturus larvae and adults). Whenever a test has been made, it has almost always been found that the presence of the eyes is not necessary for light perception and the few exceptions may have been due to the effects of experimental operations.

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[1] Unless, perhaps, it is very bright (e.g. sunlight), when they move away

The occurrence of dermal photosensitivity among widely separated Anura and Urodela, in fish (Payne, 1907) and in the 'primitive' cyclostomes (Parker, 1905; Young, 1935) strongly suggests that the skin has in the past played an important part in provoking and directing the movements of lower vertebrates in relation to light. Although, when the eye is present, its more rapid and finer sensitivity is the effective agency in influencing behaviour, it seems likely that when the eye is degenerate (as in Proteus and lampreys) or is removed by operation or masked (as in most experiments on Amphibia) the older, slower system of dermal photoreceptors, presumably somewhat impaired by many generations of desuetude, is found still to be functional.

In studying the behaviour of Proteus in the light as a means of producing aggregations in the dark, i.e. as orientations, we are confronted by apparently well marked differences between reactions at high and low intensities of stimulation: the shock reactions of photophobotaxis, dependent on photoreceptors in the head and the apparently, but not truly, random movements provoked by stimulation of the general cutaneous system of receptors over the rest of the body. The turning response which forms the basis of the first type of behaviour is gradually replaced, as the intensity of stimulation of the head is decreased, by the more indefinite restlessness elicited by stimulating the body at any, and the head at low, intensities. It seems not improbable that there is no essential difference between 'photophobotaxis' and 'apparent photokinesis' and from it; but the evidence here is not sufficient to support a clear assertion on this point.

that the shock reaction is only an extreme manifestation of a general response to light.

The turning response bears a striking resemblance to the early swimming movement of Amblystoma larvae, as depicted by Coghill (1929, Fig. 5). This is the first truly locomotor activity of the animal and it is not till some time later that the limbs differentiate and their local reflexes are developed. It is on these more recently organized responses to weak stimuli that Proteus depends in making small adjustments to slight changes in its environment. The location in the head of the receptors associated with the sudden, violent, and decisive turning response is of obvious adaptive significance, but it is important to note that these adaptations of behaviour in the light are typical of a bottom dweller in lakes and streams and are quite meaningless underground, where there is no light.

Negative phototropism' is often ascribed to cavicolous animals. It is rightly supposed that, for them, light is a signal of danger, and it is concluded that their reactions to light have evolved as adaptations which keep the animals within the safety of their dark homes. Proteus is a good example about which such misconceptions can arise. Familiarity with its native caves suggests important objections to the traditional interpretation of its behaviour as adaptive.

(1) In collecting Proteus from caves between Istria in the north to Vjetrenica pećina in Herzegowina—i.e. throughout its longitudinal range—I found not a single habitat in which the animals could have reached the cave entrance in circumstances which could have allowed it to turn back. It is only in times of flood that the subterranean waters of the caves reach the mouths, and then the animals are washed out in the torrential outpouring of the waters (Hawes, 1939). Against this general calamity, Proteus, the largest and strongest of European cave dwellers, has no protection. When the waters sink again and the restored calm provides conditions in which the animal's light reactions might be effective in directing its orientations, the cave mouths are inaccessible to aquatic organisms.[1]

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[1] The peasants of Slovenia, Kroatia, and Herzegowina are well aware of

(2) The collector finds comparatively few specimens in caves from which, at flood times, great numbers of Proteus are washed out, so that the peasants collect them for pig food or to sell to zoologists. There is no doubt that the great majority of Proteus are confined to depths of the caves inaccessible to spelaeologists, who find only exceptional wanderers. Proteus lives normally in complete darkness, and only reaches the light in conditions such that its responses are useless against the overwhelming force of the floods.

(3) The last point follows from the first two: Proteus lives all its life in the dark; it is not possible for it to have evolved means of avoiding the light. It is more reasonable to suppose that the light reactions are survivals from a period of epigean existence.

V. Acknowledgements.

Part of this work was done and the animals were collected when I held a Layton Studentship in Science at the University of London, King's College. I record my thanks to the authorities of the College for their help and for grants to cover travelling and other expenses.

I should like to express my gratitude to the following in addition to those mentioned in the text: to Professor Jovan Hadži and Dr. L. Kuščer for their assistance in Jugo-Slavia; to Professor C. M. Yonge and his staff for help in the Mendip caves; to Professor James Gray, F.R.S., for hospitality at the University of Cambridge, by which my stock of animals was saved from extinction; to Mr. K. Williamson and Mr. Denys Kempson for taking the cinematograph films and photographs respectively.

Professor H. Graham Cannon, F.R.S., kindly read and criticized some parts of this paper.

the value of Proteus and they are familiar with the caves of their districts. Any locality in which the animal approached the cave mouths and emerged on dark nights would be exploited at once as an easy means of collecting. Inquiry at the University of Ljubljana and among peasants revealed the existence of only one such place; as expected, it was used as a collecting point. If other such points existed, they would certainly be known, as the alternative means of collecting are uncertain and much more difficult.

My most sincere thanks are due to Professor D. L. Mackinnon, under whose direction this work was initiated, and to Major G. R. de Beer, F.R.S., for help and advice on many occasions.

VI. SUMMARY.

The structure of the eye has been reinvestigated. Development is arrested at the secondary optic vesicle stage, though growth continues for some time afterwards. The lens does not complete its differentiation; it usually disappears in adult animals, and is replaced by a proliferation of epithelial cells from the iris margin. If the lens persists this proliferation is inhibited. These facts are explicable on the assumption that the normal potencies of the iris margin are realized when the inhibitory action of the lens is removed, but, in Proteus, the typical dominance of the dorsal region of the margin is lost, so that the organization of the proliferated cells into a morphogenetic field does not take place.

The visual cells of the retina stop their development at about the time when rod and cone primordia first become distinguishable.

The optic nerve, greatly reduced, persists throughout life.

The vascular supply to the choroid is maintained through a minute vessel representing the ophthalmic artery.

The relations of the nerve and artery to the trabecula cranii are those of a typical urodele embryo and, in this respect, development in Proteus is arrested at a slightly earlier stage than in Necturus.

The development of the extrinsic eye muscles is arrested before their adult arrangement is achieved and before the M. rectus internus appears.

The skin above the eye is unmodified and the accessory cornea of Schlampp does not exist.

It is argued that the condition of the eye and some other structures in the orbit is just as much the effect of neoteny as such typically embryonic features as the skin and gills. The general tendency to neoteny was present when Proteus first adopted a cavernicolous life and was able to extend to the organs of vision when their function became unnecessary.

Proteus is sensitive to stimulation by light and responds to it by movement. The eyes are unnecessary for light perception.

Stimulation of the body and tail at any intensity and of the head at low intensities produces slight movements of adjustment and, if repeated, may lead to movements of translation.

Stimulation of the head at high intensities leads to a violent and sudden turning response after a latent period of reaction, dependent, within limits, on the intensity of stimulation.

The active factor of the stimulus is intensity, not direction.

The reactions at high and low intensities and the types of orientations they produce ('photophobotaxis' and apparent 'photokinesis') are possibly not fundamentally distinct. The subjective impression of movement at random below the threshold for shock reactions is erroneous; the movements are controlled, but the basis of control has not been discovered.

The light responses are not adaptations developed in relation to life in caves, in which they are never used. They are survivals from a period of epigean existence, and may have played a part in the colonization of caves by Proteus.

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EXPLANATION OF PLATE 1.

Fig. 1.—Photomicrograph of a transverse section through the eye and optic nerve of Proteus anguinus. $10\mu \times 75$. *Ao*, ophthalmic artery; *Bc*, buccal cavity; *Mlmd*, M. levator mandibulae anterior; *Mri*, M. rectus inferior; *Nld*, nasolachrimal duct; *T*, trabecula cranii; $V_1 b$, ventral terminal branch of the deep ophthalmic nerve; *VIIso*, superficial ophthalmic nerve.

Other structures shown, but not labelled, include M. rectus superior, just above *Mri* and passing dorsally to the optic nerve; two branches of *Ao*, one at the dorsal end of the superior rectus muscle and the other close to the sclera, about half an inch above the optic nerve; vena capita lateralis, touching and to the right of the guide line of *Mri*; maxillary nerve, above *Nld*; lateral terminal branch of the profundus, above *Ao*, and mesial terminal branch of the same, between *T* and *Mlmd*.

Fig. 2.—Photomicrograph of a transverse section of the midbrain of Proteus anguinus, to show the entry of the optic nerve. $10\mu \times 100$. *Ac*, cerebral artery; *Ao*, ophthalmic artery; *P*, orbital wing of parietal bone; *Psph*, parasphenoid bone; *T*, trabecula cranii; *Vcl*, vena capita lateralis; *II*, optic nerve; V_1, deep ophthalmic nerve.

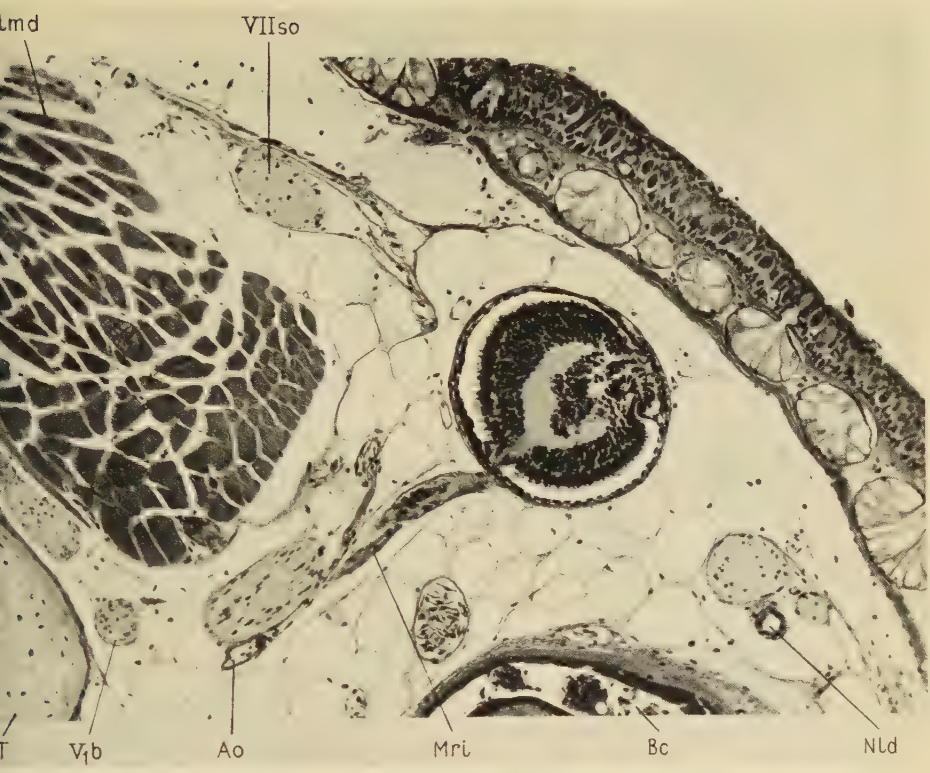

FIG. 1

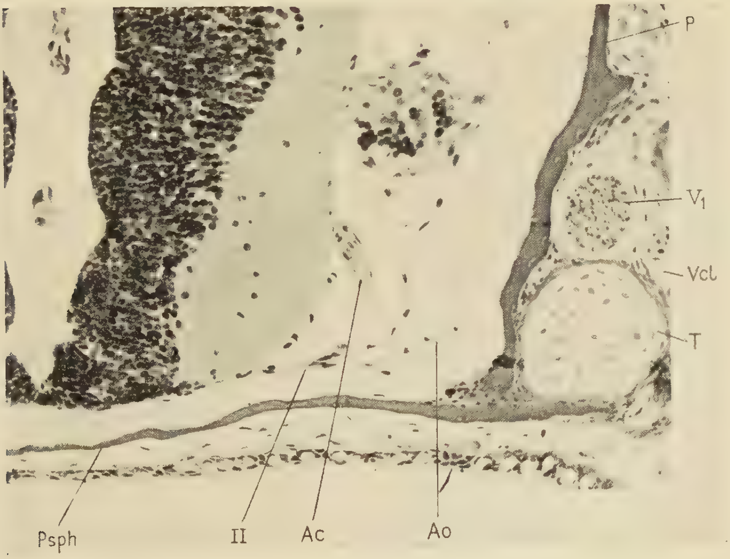

FIG. 2

R. S. Hawes

The Anatomical Organization of the Nervous System of Enteropneusta.

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With Plates 2–8, and 1 Text-figure.

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INTRODUCTION.

THE zoological interest attached to the Enteropneusta stems from the suggestion made by Bateson in 1885 that these worm-like creatures are actually chordates and that they occupy a strategic position as the most primitive of the invertebrate relatives of the vertebrates. In spite of an active interest in their morphology, the animals remain far from well known. They are commonly designated balanoglossids after the earliest well-known genus, and comprise a highly homogeneous group of five to six dozen species divided into twelve genera and three families.

Our knowledge of the nervous system has grown up largely through a series of taxonomic and morphologic studies. The nervous system was first identified by Spengel in 1877. Kowalevsky in 1886 had designated a 'Kopfganglion' in the base of the proboscis, but his structure turned out to be the proboscis complex, including 'notochord', heart, cardiac vesicle, and glomerulus. The accounts published by Spengel in 1877 and in his monograph of the group (1893 and preliminary communication, 1884) stand to-day, almost without modification or addition, as the sum of our knowledge of this system. Spengel recognized a thin layer of 'Punktsubstanz' at the base of the 'Epidermis' everywhere over the body. (The integument is a pseudo-stratified, columnar, ciliated epithelium immediately underlain by the body muscles.) The basi-epithelial plexus he considered to consist of some kind of sensory cell and some kind of associative cell and nerve-fibres. Thickenings of this layer in certain regions he described as follows. A mid-dorsal and a mid-ventral longitudinal cord in the trunk region are joined by a circular connective at the junction of this region and the collar. The ventral cord ends at this level but the dorsal cord is continued anteriorly as the collar nerve-cord, which leaves the surface and plunges into the coelom of the collar (Pl. 2) to traverse it as a solid or a hollow strand (the 'Kragenmark'). If it is solid, there are usually to be found small, unconnected cavities ('Markhöhle') scattered through it. Some species were described as having unpaired mid-dorsal extensions of the collar cord by which it communicates with the overlying epidermis. Giant nerve-cells were found in this cord. It returns to the surface at the anterior end of the collar and becomes continuous with the thin nerve layer of the outer epithelium of the collar and the deep layer on the dorsal side of the proboscis stalk. At the anterior end of this stalk or peduncle, where it is continued into the proboscis, the thickened nerve layer extends down the sides and even ventrally, constituting the so-called anterior nerve ring. This latter in turn is continuous with the general plexus under the proboscis epithelium.

Bateson (1884, 1885, 1886*a*, 1886*b*) described the development of some parts of the nervous system and submitted classical phylogenetic speculations. Koehler (1886) and a long series of authors after him described new species and showed in what points variation was to be found within the group. Schneider (1902), Hilton (1919), Clayton (1932), and de Renyi (1934) each added a few histologic details, but most of the observations in the last forty years have been in the nature of gross morphology, both because these are the important taxonomic and phylogenetic aspects and because finer details were not demonstrable. The first study of the nervous system in life was made by Crozier (1915, 1917), who reported a few observations on chemical, mechanical, and photic irritability and phosphorescence. Hess (1931, 1936, 1937, 1938) exploited the photic reaction in a series of experiments on whole animals and fragments of various constitution and applied specific nerve stains for the first time to this group. Cary (1933) successfully cultured tissues of one species in vitro; he mentions nerve-cells as the first cell-type to show activity, elongating and sending out processes in the same manner as do those of warm-blooded animals. Bullock (1940) offered an analysis of the physiologic organization of the nervous system based on simple experiments and considerations of natural history. An anatomical study of the giant fibre system, made as an outgrowth of the present investigation, has been published (Bullock, 1944). The admirable monograph recently completed by van der Horst (1927–39) brings together all available information on these organisms and is of great aid in the study of any phase of balanoglossids.

The present investigation was undertaken because of the conspicuous lack of understanding of the balanoglossid nervous system from a neurologic standpoint. Virtually nothing is known of the system except the gross anatomic facts outlined above. The motivating interest in this investigation has been in the neurologic pattern or organization, the nerve-cell types and their relations and the general level of complexity achieved.

It is a pleasure to acknowledge my debt to Professor S. F. Light, under whose guidance the work was pursued, for his generous and stimulating interest in every ramification of the problem. A number of individuals and institutions have kindly made loans or gifts of material used in this study. To these, as well as to those who have aided me in numerous ways in the collection of living enteropneusts, I am greatly indebted. I wish particularly to mention the generous assistance of Mr. J. E. Gullberg, who gave persistent and expert aid in all microscopical, mechanical, and photographic problems.

The work was done for the most part at the University of California, but further observations were made at Yale University where much material assistance was received from the staff of the Section of Neuro-Anatomy.

Materials and Methods.

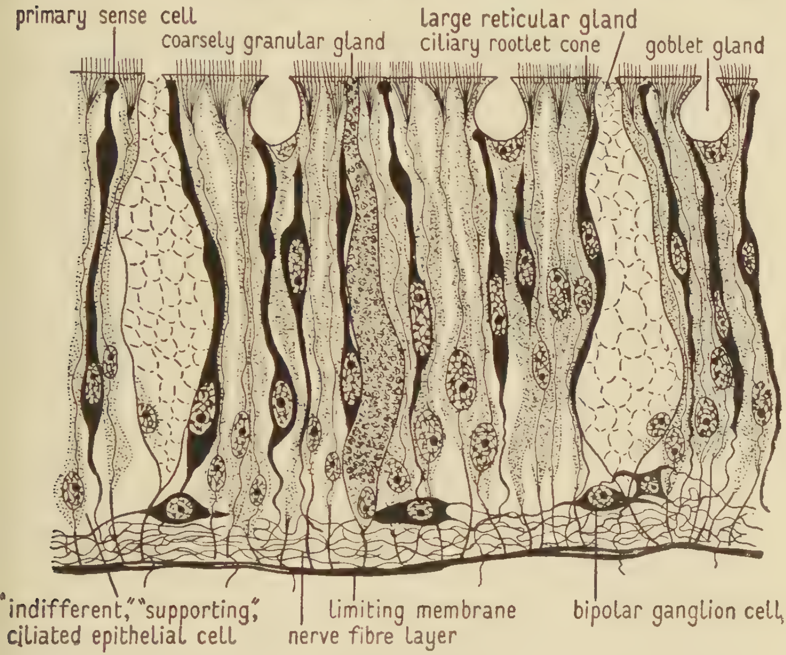
An extensive series of species, representing nine out of twelve known genera, plus one manuscript genus, has been studied. A variety of general and special technics has been employed. These are described elsewhere (Bullock, 1944). The descriptions to follow are chiefly based on Saccoglossus pusillus (Ritter), prepared (1) cytologically, (2) with protargol impregnation, and (3) with intra vitam methylene blue.

Neuro-anatomical Description.

The nervous system in Enteropneusta occupies its primitive position within the superficial epithelium of the body. The epithelium of balanoglossids is extraordinarily highly developed. It is typically of a complex pseudo-stratified type. The cell outlines are vague, but the crowded nuclei indicate a densely packed tissue. Although the epithelium in various regions and species is highly diversified, the aspects shown in Text-fig. 1 and Pl. 6 are more or less typical. It will be seen that the 'Punktsubstanz' or nerve-fibre layer is localized at the base of the epithelium but that the nerve-cell bodies are not separated from non-nervous cells. A brief consideration of certain non-nervous elements is necessary, therefore, before describing the nerve-fibre layer, nerve-cell bodies, and local differentiations.

Non-nervous Elements.

The most conspicuous formed elements in the epithelium are certain heavy fibres, oriented uniformly at right angles to the plane of the epithelium and attached at their innermost ends to the limiting membrane. The staining affinities of these fibres are, with almost all methods, diametrically opposed to those of nerve-fibres. They appear to be derived from the inner ends of the ordinary ciliated epithelial cells. This is well seen in teased or macerated preparations and in certain methylene blue stains (figs. 26, 27, Pl. 8). The degree of development of these epithelial cell feet varies from the extreme of simplicity, where the



TEXT-FIG. 1.

Diagram of the epithelium of Saccoglossus.

epithelium is a low, simple, columnar layer and the broad basal ends of the ciliated cells rest directly on the limiting membrane, to a level of complexity represented by long, discrete, formed fibres traversing the nerve-fibre layer (fig. 10, Pl. 4) in regions where the epithelium is deep and crowded. The significance of these fibres is probably that of a supporting tissue. Similar radial supporting fibres are present in the deeper epithelia of phoronids and echinoderms and some other groups of invertebrates.

In certain regions of some species I have seen what appears to be direct continuity between these fibres and the intracytoplasmic fibres formed by the fusion of the ciliary rootlets.

In these structures, if anywhere, is represented the neuroglia of enteropneusts. If neuroglia may be defined as any differentiated supporting elements in the nervous tissue, then the term may be applied to these radial 'Stützfasern', different as they are from the usual invertebrate glia. Moreover, we have here the most primitive form of neuroglia to be found anywhere, for the cells which produce the supporting fibres are at the same time fulfilling the functions of ordinary ciliated epithelial cells. The same animal, indeed, presents all transitions to a condition so simple that structures differentiated for support are lacking and the term neuroglia is inapplicable. Even in the regions of highest elaboration the radial fibres do not achieve the complexity I have observed in the epithelia of phoronids and echinoderms.

The variations in elaboration of radial fibres between regions are correlated closely with variations in the development of the nerve-fibre layer. Exemplifying this correlation and indicating the highest level attained by the radial fibres in these organisms, is the condition found in the nerve-cords. Here the supporting fibres show a tendency to alinement. Rows of them, oriented in a direction corresponding to that of the nerve-fibres, cut the punctate substance into fascicles. However, the rows are loosely formed, uneven, and rarely extend as much as 75 μ, so that the fascicles are defined for only a short distance before splitting or anastomosing. This alinement is correlated not with bulk of nervous tissue but with unidirectional orientation of nerve-fibres and thus indicates regions which are more in the nature of conduction tracts and less in the nature of neuropiles.

There occurs in the epithelium of certain species or genera (in Balanoglossus and Ptychodera, but not in Saccoglossus and most others) a layer of fibres parallel and immediately peripheral to the nerve-fibre layer (fig. 17, Pl. 6), which we may call the horizontal fibre layer. It was regarded by Spengel (1903) and van der Horst (1924) as a cellular layer, the latter author suggesting in 1927 (1927–39) that it may be a layer of nerve-cells. The evidence from the present studies indicates a non-nervous character and, moreover, a probable common origin with the radial fibres. It is not regularly correlated with any feature of the nervous system, and must be left for the present purposes as a peculiar local specialization of the supporting tissue of the epithelium of some forms.

Of more importance here is another supporting structure, the limiting membrane. This is a continuous sheet underlying the superficial epithelium throughout the body, forming the mesenteries and blood-vessels, extending into the linings of the gut and gills, and locally thickened to form the skeletal masses of the proboscis, collar, and gills. Previous study of this structure has been centred around the question of its origin. Its importance in the present connexion lies in its conspicuous, sharp, and ubiquitous separation of the nervous system from the muscular, a separation which on physiologic grounds cannot be absolute.

The limiting membrane is poorly seen in ordinary double stains, but stands out brilliantly in suitable triple stains (Mallory's, Masson's). It appears (Text-fig. 1) in the best fixed and stained preparations as an uninterrupted, homogenous membrane from one-half to several micra thick, consisting of two distinct lamellae but otherwise structureless. It may be folded or creased, and in oblique sections may appear discontinuous (fig. 16, Pl. 6) owing to the conspicuous creases and edge-views and very inconspicuous flat-view. Many preparations will present oblique sections in which no sharp boundary between epithelium and muscle can be made out. But in general it appears from careful observation of the best preparations that the limiting membrane is always present, continuous, and uninterrupted. Any structures communicating between epithelium and underlying tissues must pierce the membrane. But by all ordinary (critical) methods nothing can be seen to penetrate it (Pls. 3, 4, 5). The best cytological preparations have been repeatedly searched and no structure or fibre has been seen definitely to cross this boundary. Of course, it is not to be expected that single nerve-fibres would be seen in cytological stains, but the significant result of these observations—with stains which show the membrane well, whereas selective nerve stains do not—is that whatever does cross it must be of the order of single nerve-fibres. No 'nerves' or even small bundles have been found to pierce it.

Fibrous Matter of the Nervous System.

The stratum of punctate tissue at the base of the epithelium, shown in all the figures, represents the most conspicuous, indeed, the only obvious constituent of the nervous system of balanoglossids. It is found in all but the thinnest regions of the superficial epithelium and the more specialized levels of the gut, and masses of nerve-fibres are found only in the epithelia. No internal sub-epithelial masses or cords are definitely known. The punctate tissue consists of fine, horizontally oriented nerve-fibres and the heavier radially arranged supporting fibres already described. Its importance is twofold. In it are developed the great nerve-cords, indeed, all nervous communication between parts, adjacent and distant; and in it are located, in all probability, the synapses or whatever form of physiological continuity between neurons exists in this group.

As is seen in the photographs, the layer is rather sharply defined on its outer as well as its inner surface. It is characterized, with limited exceptions, by the lack of differentiations within its thickness; there is no stratification; fibres passing in one direction are not segregated from those passing otherwise. It is significantly simpler in organization than the neuropile of most other invertebrates.

The intimate structure of the nerve-fibres themselves (Pls. 7, has been best observed in preparations stained with methylene blue (intra vitam), protargol, and osmic 'acid' by the Mann-Kopsch Golgi body method. They are thin and rather uniform in diameter; at least they lack marked irregularities. The thorny projections and warty excrescences of some nerve-cell processes are absent. The degree of sinuosity is characteristically gentle, not acutely angular. In all these respects, all nerve-fibres of enteropneusts resemble in general the axon of vertebrate neurons as opposed to the dendrites, a resemblance shared by the nerve-fibres of coelenterates, platyhelminths, and other lower invertebrates. I have not made out neurofibrils except in the giant fibres (Bullock, 1944) and in the cell-bodies of methylene blue preparations (Pl. 7). De Renyi (1934), in a preliminary note, reports the observation of neurofibrils in living cells of Ptychodera by micro-dissection; and Hess (1937) figures them in the giant cells of Saccoglossus. All nerve-fibres, except the giant fibres, appear to be alike. No differentiations of axon and dendrite, of 'Zellenfortsatz', 'Markfortsatz', and 'Nervenfortsatz' (of many invertebrate unipolar neurons) have taken place. Nor have boutons terminaux or other specialized endings been seen.

Branching occurs, and in this respect also the enteropneust fibres suggest the axon of heteropolar neurons. The branching is in general dichotomous and relatively infrequent. The branches are, like collaterals, equivalent in size to the main fibre, if indeed either one can be distinguished as the main fibre. Hilton (1919) apparently had a different conception, for he considered the fibre matter of enteropneusts to consist mostly of short lateral twigs of the main processes. He further regards this condition as more highly developed than that which he found in echinoderms, whose fibre matter consists mostly of 'strands from cells', but it is not clear how he was able to make out any of these details, not having used special stains. Our knowledge of the echinoderm nerve elements is as meagre as for the present group, and no one has described the fibrous matter from selective nerve stains. But my own examinations of cytologically prepared material of Leptosynapta, Pisaster, and other genera have revealed no less complexity and, in fact, greater heterogeneity of fibre structure and arrangement than in balanoglossids.

No indications whatever have been found of sheaths around the nerve-fibres.

There now arises the question of the nature of the anatomical relations between neurons. Is the nervous system of balanoglossids synaptic or asynaptic, or a mixture of these conditions?

Unfortunately I am unable to give a satisfactory answer. Good methylene blue stains are the most reliable preparations for this purpose, but my results with this stain on Saccoglossus pusillus and Saccoglossus kowalevskii have never been complete enough to justify an exclusive generalization. Such stains as were obtained never showed a nerve-fibre which could be traced to a neuron in both directions (i.e. an anatomically continuous nerve-net), but did show many long and delicate fibres passing, apparently independently, among each other, through the plexus. These pictures, per se, would not seem to be more than suggestive. But their resemblance to such figures as those of Bozler (1927), on which Hanström (1928) places the greatest weight as demonstrating synaptic relations in coelenterates, and their complete divergence from figures offered by proponents of the theory of continuity in the nerve-net (listed and reviewed in Hanström and in Bozler), lend some support to an interpretation of these observations on enteropneusts in favour of the synaptic theory.

Sections of protargol material, cut parallel to the plane of the nerve-fibre layer, show that plexus beautifully, although thin sections impose limitations on the tracing of fibres and on the interpretation of apparently free nerve endings. Here, again, after careful search. I am unable to rule out the possibility that anastomoses of fibres occur, but on the other hand, neither was it possible anywhere to demonstrate a case of anastomosis, as opposed to mere contact or crossing of fibres. The fibres could often be followed for long distances, and the rich plexus studied fibre by fibre, the general impression always remaining that any anatomical continuity that did exist must be very rare. The only opinion to be found in the literature is that expressed by Hess (1937), who believes that no true nerve-net but '. . . a plexus of . . . neurons . . .’ is involved in Saccoglossus kowalevskii. Neither interpretation of the structure has been demonstrated. At present, however, the evidence seems to lean towards the assumption of a discontinuous, synaptic condition.

It is of some interest to determine where in the nervous system synapses or other forms of physiologic connexion between neurons occur. In successively higher groups of animals a striking process of concentration of synaptic fields is to be noted. In the lowest groups possessing nervous systems the connexions between neurons are scattered throughout the system; but early, beginning with the lowest worms, these areas of interneuronal junctions are restricted to certain parts of the system. The indications for balanoglossids are that all or nearly all neuron-to-neuron communications occur in the nerve-fibre layer, and that such junctions are unlocalized within this layer but are scattered everywhere over the body. This conclusion is strengthened by the evidence, described in the next section, of wide distribution of nerve-cell bodies, and even more by the physiologic evidence of local reflex arcs and neuromuscular autonomy of small pieces (Bullock, 1940).

The problem of communication across the limiting membrane between epithelial nervous system and sub-epithelial muscles has already been introduced by the statement that nothing can be seen to cross this boundary in critical preparations by ordinary methods. Remarkably enough, even in successful protargol stains, such as clearly show individual nerve-fibres, nothing can be traced from the epithelium into the muscles. Again, great care in the use of sections and adequate optical resolution is necessary, for many confusing and uncertain appearances are encountered. But while it is true that the general integrity of the limiting membrane is emphasized in these preparations, there have been encountered many situations where fibres of the nerve-fibre layer turn inwards and apparently penetrate the limiting membrane, though they have not been followed definitely into the muscles in any case. These pictures are the only visible evidence of crossing I have found and they are unsatisfactory. Although the problem of the shallow focal plane is here acute, photographs of such situations are reproduced in figs. 20 and 21, Pl. 6, as the only objective anatomical evidence offered, here or heretofore, for the existence of a motor-nerve supply. It is interesting to recall that in no group possessing a superficial nervous system and no internal ganglia—for example, coelenterates, echinoderms[1], phoronids—have the motor connexions been established. The negative statement that nowhere in the body of the balanoglossid is there to be found a localized inflow of nerve-fibres from the epithelium to the muscles is more easily established than the positive suggestion given above. These two arguments, however, converge towards the presumption that the motor-nerve supply of enteropneusts is a generalized, diffuse, fibre-by-fibre crossing of the limiting membrane, a condition that would account for the degree of local reflex autonomy found experimentally (Bullock, 1940).

Nerve-cell Bodies.

By ordinary staining methods nerve-cell bodies are not with certainty distinguishable from other cells of the epithelium among which they are scattered. Only the giant cells and the larger ganglion cells of the collar cord are recognizable in such preparations. But in any section adequately preserved to show the infraciliature, glands, and nuclei, strongly presumptive evidence of nerve-cells is easily obtained.

First, it must be said that extended examination has indicated the probability that three cell-types, nerve-cells, gland-cells, and ciliated cells account for the entire epithelium. No other cell-type has been recognized in the animals studied and none is reported in the literature. But one possibility has been suggested from the histology of certain other invertebrate groups, namely, that undifferentiated reserve cells may be present throughout the epithelium. A special search for these was in vain. Further evidence that there are few, if any, such cells is given on another page. Second, it seems safe to assume, from study of the simpler epithelia and maceration preparations, that all the cell-types are normally uninucleate.

Now, we may obtain the total number of cells in any portion of the epithelium by counting nuclei. The total number of ordinary epithelial cells may then be obtained by counting the conspicuous converging bundles of ciliary rootlet fibrils. Likewise, we may count the gland-cells. True, there is the possibility of error in this count, due to the presence of collapsed glands; however, several considerations render it likely that the error is not large. Thus, individuals, treated in various ways designed to encourage secretion of mucus just before fixation are not greatly different in respect to numbers of gland-cells visible from specimens fixed by rapid freezing in liquid air. The existence of many empty but uncollapsed glands, and the ease with which the nuclei that belong to the commonest type of gland (small, peripheral goblets) may be recognized, likewise argue that this error is not large. If the number of ordinary epithelial cells plus the number of gland-cells be subtracted from the total number of nuclei, the remainder should represent the number of nerve-cells in that portion of epithelium. Such counts have proved more than instructive and little short of astounding.

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[1] A recent paper by J. E. Smith (1945) promises a description of these elements in asteroids in a forthcoming issue of the Philosophical Transactions.

Thus, an extent of lateral trunk epithelium, bounded by deep grooves, in an 8 μ section measured 81 μ in length, contained 167 nuclei, 50 rootlet bundles, and 20 glands. No significant estimate of the error in gland-cell count is possible, but even if it is 100 per cent. or more the small figure involved does not change the general result materially. There remain a number of nuclei—65 to 120—exceeding that accounted for by any other cell-type, which would seem to belong to nerve-cells. A portion of proboscis epithelium in the same section, 74 μ in length, not encroached upon by grooves, contained 284 ($\pm$5) nuclei, 50 ($\pm$20) rootlet bundles, and 12 gland-cells. About two-thirds of all the cells in this area of the proboscis epithelium apparently are nerve-cells.

These counts vary in a highly significant manner from one part of the body to another. The specific results will be mentioned below in the regional survey. But some generalizations may be made here. (1) The number of ciliated epithelial cells per unit of surface area is approximately the same everywhere on the body. (2) The number of gland-cells varies greatly but is never more than a small proportion of the total number of cells. (The situation in the collar epithelium may form an exception; here the glands cannot be counted owing to their richness and indefinite outlines, but I believe their nuclei number fewer than those of the ciliated cells.) Thus, a nearly constant figure represents the number of non-nervous cells per unit of surface area anywhere in the epithelium. The difference between this figure and the total nuclear count varies greatly—from zero to three or four times the 'constant'. (3) This variation corresponds closely to the thickness of the nerve-fibre layer, to the threshold of irritability in life, and to the abundance of one of the two chief types of nuclei. This is the chromatin-rich cigar-shaped form as opposed to the round or oval lighter-staining nuclei characteristic of the ciliated cells. Thus, in the sensitive epithelia of the proboscis, the difference between ciliated plus gland-cell count, and total nuclear count is great, the nerve-fibre layer is thick (the epithelium over the nerve-cords forms a special case), and dark cigar-shaped nuclei are numerous. In the abdominal areas between glandular islands few or no gland-cells are present, the nuclear count is so close to the ciliary rootlet-bundle count as to be within the probable error of that figure, no nerve-fibre layer is visible, and no cigar-shaped nuclei occur. These correlations would hardly be expected of undifferentiated reserve epithelial cells and constitute, therefore, presumptive evidence that no considerable number of such cells exist. The hypothesis may then be suggested that these great variations in cell population of the enteropneust epithelium are due primarily to variations in the abundance of a single cell-type, and that this is probably a sensory nerve-cell.

Turning now to special nerve stains, the neurons most consistently demonstrable in both methylene blue and protargol preparations are just such elements—primary sense-cells (figs. 18, 19, Pl. 7; figs. 22, 23, 25, Pl. 8). These are elongate, upright cells, that is, perpendicular to the plane of the epithelium. One process, a typical nerve-fibre, extends from the inner end of the cell into the basi-epithelial plexus. The outer end of the cell-body is much constricted and tapers until, near its termination just below the surface, it often exhibits a slight swelling. A bristle-like projection is sometimes apparent, often not. The nucleus is well shown in protargol stained sections and is usually of the elongate, cigar-shaped type which is most abundant in the zone just peripheral to the deep-lying round and oval nuclei. Although slight individual variations are common, I was unable to subdivide these cells into morphological types. There seems to be but one kind of sensory cell in the balanoglossid. The primary sense-cells are much the most numerous neurons encountered (absolute figures on their abundance are not possible with these methods). They account for the majority of fibres making up the basi-epithelial plexus.

The other nerve-cell types found in the epithelium may collectively be termed ganglion cells, although no true ganglion exists in the Enteropneusta. They are all lodged in that zone of the epithelium immediately peripheral to the nerve-fibre layer (or the horizontal fibre layer when present), that is, they are not scattered in the plexus but are sharply segregated from it. Occasional exceptions are found, particularly in the peduncle of large species, where a significant number of nuclei may be seen within the thick nerve-fibre layer. But this is rare elsewhere. At present no distinction can be made between intermediate and motor neurons; both are probably present in the epithelium, side by side. Ganglion cells of bipolar, multipolar, and unipolar varieties occur, but the last named are confined to the collar nerve-cord and adjacent levels of other nerve-cords. Bipolars are the most common (figs. 28, 29, 30, Pl. 8) and for the most part are rather large (15 to 20 μ long) with round or short oval nuclei and processes arising abruptly from two sides of the cell. A few smaller spindle-like cells have been seen (under 10 μ). No differences were noted between the two poles of the cell. In the cell-bodies of some individuals in methylene blue preparations, clearly defined sinuous threads were visible which suggest neurofibrillae. The typical orientation of bipolar neurons is parallel to the nerve-fibre layer.

In the abdomen, where the best methylene blue stains were obtained, a very few multipolar neurons were found (fig. 31, Pl. 8). These were simple isopolar cells with large bodies, processes leaving each of the corners, and delicate fibrillae filling the cell except for a circular space in the centre, which doubtless accommodates the nucleus.

Unipolar neurons have not been identified with any certainty in the general epithelium but occur clearly in the nerve-cord of the collar. No methylene blue pictures of them have been obtained, and only fragmentary pictures of the cell-body with protargol. But they are often relatively large and can be found in any cytological preparation. These cells vary in outline but usually have a rounded end on the side nearest the surface of the epithelium and a single process emerging from the opposite side. They have a fairly large proportion of cytoplasm and a round or short oval nucleus of average size, i.e. 3–5 μ in diameter, or larger, even reaching 7 μ to intergrade with the giant cells.

The remarkable giant cells and fibres have been described elsewhere (Bullock, 1944).

Regional Description.

Proboscis (figs. 4, 5, Pl. 3; fig. 11, Pl. 4; fig. 17, Pl. 6).—Two general features characterize the nervous system of the proboscis. It is comparatively well developed and is comparatively uniform over the entire organ. The nerve-fibre layer is here thicker than anywhere else in the body, excepting the nerve-cords. Its uniform thickness, anterior to the peduncle and base of the proboscis, is striking. No local specializations, dorso-ventral differential, or thickened spot at the active anterior tip can be demonstrated. In some species an exception of questionable significance is formed by the occurrence of a mid-dorsal thickening. Assheton (1908) noted such a thickening in Saccoglossus serpentinus, also Hinrichs and Jacobi (1938) in Saccoglossus pygmaeus, and van der Horst (1930) in Saccoglossus otagoensis and Glossobalanus ruficollis, the last-named species having also a mid-ventral thickening. A mid-dorsal differentiation is commonly present in Saccoglossus pusillus, but not in my specimens of Saccoglossus kowalevskii. In the other species I have examined only occasional suggestions of a cord are encountered. They either extend through a few sections only or are not definite enough to merit recognition. It may be said therefore, that there are essentially no nerve-cords in the proboscis in most enteropneusts.

Whereas the fibre layer of the proboscis epithelium of any individual—as represented by sections—has a rather definite thickness, that measurement varies considerably between specimens of the same species. This is noteworthy, in connexion with the common practice of including in taxonomic descriptions a figure for the depth of the fibre layer and another for the depth of the entire epithelium, or a ratio of the two. I find that in the same species these two measurements may vary apparently independently. Such ratios have little or no significance therefore. Doubtless, the state of the animal at the time of fixation and other technical factors are largely responsible for the apparent variation. It is true that the measurements of the nerve-fibre layer vary less than do those of the epithelium as a whole; still, the variation is almost as much within as between species.

If the hypothesis advanced in the previous section is correct, that the excess of nuclei over ciliated cells and gland-cells represents the number of nerve-cells in the epithelium of the balanoglossid, then for certain preparations of Saccoglossus pusillus the nerve-cells number approximately 37 per 100 square micra of surface. This figure means little as an absolute quantity, depending as it does on the state of the glands at the time of fixation, the contraction of muscles, and so on, but it is significant relative to the figures given below for other regions which were obtained in the same preparations. (These counts were made on stretches of epithelium 70–90 μ in length in 8 μ sections, thus involving several hundred square micra of surface and several hundred nuclei, the latter counted from camera lucida drawings.)

The directions taken by the fibres in the nerve-fibre layer are, in all parts of the proboscis except the dorsal cord of certain species, not restricted. Antero-posterior, circular, and oblique courses are equally demonstrable. I am not able to say whether there is a significant preponderance of fibres passing in one direction, but I find no segregation, for example, of outer longitudinal and inner circular elements. We have here, then, the conditions for a true nervous plexus, and the anatomical basis for the diffuse conduction found in life. But this does not imply necessarily a network of anastomosing fibres, a feature frequently identified with, but not at all necessary to, a plexus.

If differentiated sense organs, as opposed to scattered sensory cells, were to be found in balanoglossids, they would logically be expected in the proboscis. It may be appropriate, therefore, to state at this point that I have found no structures which could be identified as sense organs, either in the proboscis or anywhere else in the body, in any of the species I have had opportunity to examine.

Peduncle.—The peduncle or stalk of the proboscis (fig. 1, Pl. 2; figs. 4, 5, Pl. 3; fig. 10, Pl. 4) depends for its existence on the state of extension of the specimen; it is never easily delimited. For the present purposes the term is applied to the region beginning, anteriorly, with the first significant modification of the general condition of the nervous system of the proboscis and ending at the commencement of the collar cord. The most anterior modifications are a gradual increase in thickness of the fibre layer, but not of the rest of the epithelium; an increase in the number of primary sense-cells at the expense of gland-cells; and a regimentation of the fibres in respect to orientation. Over the base of the proboscis the thickening of the nerve-fibre layer is essentially equivalent on all sides. Here the fibres tend to be oriented circularly, forming the 'anterior nerve ring' of some earlier authors. But as the constricted portion of the stalk is reached, an inequality is developed. Thus, the dorsal and lateral sides of the peduncle show the nerve-fibre layer at its greatest depth in the entire enteropneust body, reaching 50 μ in Saccoglossus pusillus and 150 μ in Balanoglossus occidentalis, whereas the ventral epithelium has a very thin fibre layer (varying in thickness according to species and at least partly dependent on the development of the ventral keel of the skeleton). In this constricted portion of the peduncle the nerve-fibres are alined antero-posteriorly, which together with the fact that the great majority of fibres are patently not the processes of local cell-bodies but of distant ones distinguishes this region as a rather advanced form of conducting tract. I am unable to demonstrate whether it is merely a conduction path or whether it acts also in the capacity of a 'centre' or ganglion; but reasoning from the strikingly parallel orientation of the fibres, and the lack of neuropile-like plexus, I prefer, tentatively, to regard the peduncle as essentially conducting in function, funnelling fibres from the proboscis into the collar cord.

Other epithelia of the peduncle are continuous with this highly nervous region. It is of interest, therefore, to search for nerve tissue in these structures. Interrupting the peduncular fibre tract in the region of its greatest development is the proboscis pore, which places the coelom of that organ in communication with the outside. The short canal between the coelom and the pore is lined with a low, ciliated epithelium. I have never found a trace of nervous tissue here, though a few micra of continuous epithelium bring us to the greatest concentration of nervous tissue in the body. In certain species of balanoglossids, notably those of the genus Ptychodera, there occurs in the anterior wall of the buccal cavity (the posterior ventral wall of the proboscis) a 'racemose' or 'blumenkohlähnliches' organ (fig. 5, Pl. 3). This consists, essentially, of a more or less folded thin outpouching of the ventral coelomic diverticulum of the proboscis. It thus comprises two layers—the epithelial lining of the coelom and the outer body epithelium. The function of this structure is unknown. Of its nervous system I may say that the thick nerve-fibre layer of adjacent epithelia is reduced in the racemose organ to invisibility. Finally, the 'notochord' may be mentioned at this point, although it extends posteriorly into the collar and anteriorly into the proboscis. The highly modified epithelium of this hollow diverticulum is typically continuous with the lining of the buccal cavity. It is ciliated, in large part at least. In three of the species I have examined, a distinct nerve-fibre layer is present in the 'notochord', though in the majority there is none. In Balanoglossus occidentalis the 'nuchal' portion of this organ has a conspicuous plexus of nerve-fibres about 2–3 μ thick, bounded externally by a well-developed plexus of horizontal fibres. Both layers disappear anteriorly as the 'notochord' passes into the proboscis and changes its histologic character. In Ptychodera bahamensis and an undetermined species of Glandiceps, the most posterior levels of the organ, close to its opening into the buccal cavity, display a visible nerve layer, but it diminishes sooner than in the first-named species. Willey (1899) found a similar condition in Balanoglossus biminiensis. No function has been suggested for this nerve supply to the cavity of the 'notochord'.

Collar.—The superficial nervous system of the collar (figs. 4, 5, Pl. 3; figs. 13, 14, Pl. 5) is very simple. No specialized regions or concentrations have been found; the nerve-cords do not occur here; the orientation of fibres is unrestricted, that is, fibres run in all directions within the plane of their plexus; and the fibre layer does not vary greatly in thickness over the whole surface but is everywhere thin. In Saccoglossus pusillus and other species with a highly modified glandular collar epithelium, the number of nerve-cell nuclei is difficult to ascertain, because the glands cannot here be counted. However, they may be estimated. The total number of nuclei minus the number of ciliated epithelial cells (rooted bundles) is about 21 per 100 square micra of surface. This represents gland-cells and nerve-cells. There are at least a few gland-cells, but probably not more than eight (=the number of ciliated cells in this area). Probably not more than eighteen and not less than thirteen nerve-cells exist in this unit of epithelium, therefore. It is clear that the density of nerve-cells in the collar of Saccoglossus pusillus is significantly less than that in the proboscis, and about equal to that of the trunk.

Other species show considerable variation in the histology of the collar epithelium. In Ptychodera, for example, no great development of glands occurs in the collar, the epithelium being like that of the proboscis and anterior regions of the trunk. Here the significant diminution of nervous tissue characteristic of Saccoglossus does not take place, the thickness of the nerve layer and number of nerve-cells remaining very close to the level found in the proboscis. In Balanoglossus, however, the great differential between proboscis and collar nerve layers does exist, although the epithelia of the two regions are very similar. Both are highly glandular and exceptionally tall in this form, recalling the collar of Saccoglossus.

As in the coelomic pore of the proboscis, no visible nervous tissue can be made out in the epithelium of the canals of the collar pores.

The nerve-cord of the collar (fig. 1, Pl. 2; fig. 6, Pl. 3; figs. 12, 13, 14, Pl. 5) presents for examination the only internal mass of nervous tissue in the enteropneust body. This cord is commonly thought of as the central nervous system of the balanoglossid, and is the only candidate for the distinction of being called a ganglion. It exhibits a combination of characters unique among the nervous concentrations of animals and correspondingly difficult to interpret. It is simply a submerged strip of epithelium traversing the dorsal mesentery, and continuous at both ends of the collar with the superficial system in the mid-dorsal line. It neither gives off nor receives nerves (using the term to mean discrete bundles of nerve-fibres). No cellular sheath encapsulates it, but only a limiting membrane precisely like, indeed, continuous with, that bounding all the epithelia of the body. It is dorsal to the digestive tract and in some few species is hollow, which perhaps is its primitive condition. It is neither terminal nor related to important sense organs, but is stretched along the principal nervous pathway of the body. Although it has developed specialized giant cells and many smaller unipolar neurons, its fibre matter resembles a conduction tract more than a neuropile, and its cross-sectional area is far smaller than that of the peduncle and but little greater, sometimes smaller, than that of the dorsal and ventral cords of the trunk combined.

Some further anatomical features may be mentioned briefly. (1) The shape of the cord is highly variable within the same species. It is generally flattened dorso-ventrally, but is apparently subject to pressure from the surrounding musculature and changes shape freely. It, therefore, has no characteristic gross form, as do most internal nerve masses elsewhere. (2) In some species there occur median, unpaired medullary strands connecting the cord with the overlying superficial epithelium.

These so-called dorsal roots or crests will be described in greater detail below. (3) As would be expected from the epithelial character of the cord and its dorsal position, the fibrous portion is ventral and the cellular portion dorsal. But the cord is always more or less rolled up at the edges. Thus it might be expected that fibrous matter would be found on all sides of the cellular portion. This condition is rarely achieved, the fibre layer usually being undeveloped dorsally. (4) The adjacent coelom of the collar is almost empty, dorsal and lateral to the cord, but ventrally the cord is in contact with the perihaemal pockets of the trunk coelom which project anteriorly into the collar and are almost filled with muscle-fibres. Ventro-lateral to these is the often considerable musculature of the wall of the buccal cavity. Since there are no other important effector organs in the vicinity and no special sense organs, these muscles are the only organs likely to be innervated by the collar cord. They are responsible for movements of the collar, but their importance in the life of the balanoglossid is not known. (5) At the anterior and posterior ends of the cord, where it emerges to the surface, there may sometimes be distinguished deeper or shallower invaginations of the outer epithelium. These have usually been called neuropores, especially in species wherein they are continuous with an axial canal, but Spengel (1893) refers to them as 'vordere und hintere Epidermistasche' and shows them to be often independent of and dorsal to the nerve-cord itself. Willey (1899) distinguishes a 'neuropore (*sstr*)' and an 'epidermal pouch', which two invaginations may coexist in the same individual or may coincide as an '"anterior neuropore", in the wider sense'. These and later authors have shown that great variation exists between species with respect to this character. My observations emphasize the variability within a species, dependent on the state of contraction and the position of the flaring margins of the collar when the animal was fixed.

A continuous axial canal traverses the collar cord in the genus Ptychodera, many members of the genus Glossobalanus, and in one species of the genus Balanoglossus, all belonging to the family of the Ptychoderidae, and in a single species outside this family, Glandiceps malayanus of the Spengeliidae. As seen in Ptychodera bahamensis (fig. 5, Pl. 3), the lumen (axial canal) is large and unobstructed, widely open to the outside at both ends. Its lining is a ciliated glandular epithelium little or not at all modified from that typical of the outer surface. All members of the Harrimaniidae, all but one species of the Spengeliidae, and most of the Ptychoderidae, however, exhibit collar nerve-cords which are essentially solid. In all these species there are scattered through the cellular portion of the cord isolated cavities, often small (3 μ in diameter) or only potential. (One species, Schizocardium peruvianum, has been supposed on the basis of Spengel's description (1893) to lack even these cavities. I have been fortunate enough to be able to section and study the only balanoglossid known to me to have been taken from the Pacific coast of continental South America since that time, a specimen which is unquestionably of the same species as Spengel's. Numerous small and virtual cavities, recognizable by the radiating lining cells, are present in the collar cord of this specimen.)

Ritter (1930) stated that the nerve-cord of Saccoglossus pusillus was without cavities. In the numerous examples, indubitably of this species, which I have sectioned, I have never failed to find numbers of small lacunae, and they can be found in Ritter's original slides, also. Most of the lacunae are imperfect, that is, the cavity is virtual and the prominent row of basal granules does not form a closed figure, being usually open dorsally. Cilia are present, together with intra-cytoplasmic fibrils, but in relatively small number and in poorly developed state. The rootlet fibrils do not converge into cones as in epithelial cells, and the free portions of the cilia, which project into the lacuna, are tangled, giving the appearance of a non-functional apparatus. A definite, characteristically staining cuticle may be present. Gland-cells which appear to be functional may be found in the walls of the lacunae, emptying, therefore, into blind spaces.

The number of lacunae in the collar cord of Saccoglossus pusillus varies between limits of about 40 and 100. In Balanoglossus occidentalis there are several hundred.

The lacunae are distributed in most species in two longitudinal rows, one on each side of the mid-line, about evenly divided as to number, but not paired or evenly spaced.

Although the nerve-cord of the collar is essentially a tube of epithelium, the nervous portion is usually confined almost entirely to the floor; the roof is often poorly developed even as a simple epithelium. The floor of the collar cord is always thicker than the roof, and in species with scattered cavities the latter may be evident only laterally.

For convenience' sake many authors speak of the 'cellular portion' of the cord, meaning that tissue which includes the cell-bodies of the nerve-cells, the cytoplasmic portions of the ordinary ciliated epithelial cells and gland-cells, as opposed to the 'fibrous portion', that is, the nerve-fibre layer (see Pl. 5). Nerve-fibres certainly originate in the cellular region, but there is no evidence that nerve-fibres terminate in it to any considerable degree. It must be assumed, therefore, that this region is not one of synapse and functional interrelationship of neurons, but simply a concentration of the cell-bodies of neurons whose important nervous functions are discharged almost entirely in the fibrous layer. The cellular region may be subdivided into more or less clearly marked zones corresponding to the strata of the outer epithelium. A reduced ciliary apparatus and infra-ciliature bounds the lacunae. A zone of cytoplasm, chiefly of the epithelial cells, includes a concentration of pigment granules (often very conspicuous, see Willey, 1899; Spengel, 1904*a*), osmiophilic granules, eosinophilic granules, and granular mitochondria. (How many of these groups of elements are the same structures it is impossible at present to say.) Most of the nuclei of the cord occupy the zone next to the fibre layer, although minor topographic differences occur between species. The nuclei are all of the round or short oval type; none of the dark-staining cigar-shaped forms, identified earlier with primary sense-cells, occur in the collar cord. In species which possess horizontal supporting fibres in the general epithelium a layer of these elements is interposed between the nuclei and the nerve-fibres. Sometimes it is only moderately developed here, as in Ptychodera, but, again, it may be extraordinarily thick and modified in staining affinities from the same elements elsewhere, as in Balanoglossus occidentalis.

The resolution of cell boundaries is as difficult in the collar cord as elsewhere and it is not possible to count the ciliated cells. But it is apparent, on the one hand, that considerable numbers of non-nervous cells occupy the cord, and, on the other hand, that the majority of cells are nervous. No evidence of sensory cells has been encountered and, in fact, nuclei of the type characteristic of primary sense-cells are lacking. It has been impossible to separate motor and intermediate neurons. The 'ganglion' cells of the cord include unipolar and multipolar forms and perhaps bipolars, but the first named, I believe, predominate. Included in the unipolar category are the giant cells, described elsewhere (Bullock, 1944).

Finally, it should be said that the nerve-cells of the collar cord appear to be distributed uniformly through the region they occupy. No differential distribution of certain types is apparent, except for the posterior concentration of giant cells. No special 'nuclei', strata, or 'corpora' of nerve-cell bodies occur.

The nerve-fibre layer is composed of fine processes of nerve-cells and heavy radial supporting fibres. It occupies a little less than half the cross-sectional area of the cord. The nerve-fibres have the same character as in the superficial system. They are gently sinuous, rather smooth, dichotomizing, and without thorny side branches or elaborate endings. They present a conspicuously parallel orientation and run longitudinally. No real segregation, differentiation, or stratification is detectable, with the exception of the giant fibres. Van der Horst (1929) describes an outer concentration of transverse fibres, and an inner of longitudinal fibres in the posterior half of the cord in Glandiceps talaboti, but I have seen nothing of this kind in other species. Thus, the fibrous mass in the collar cord differs in no essential from the same layer in other cords; it shows no morphologic evidence of specialization nor do its fibres form a tangled neuropile.

All members of the Ptychoderidae possess one or more strands of cellular tissue which traverse the dorsal mesentery of the collar, and connect the collar nerve-cord with the mid-dorsal outer epithelium (fig. 1, Pl. 2; fig. 13, Pl. 5). These median unpaired structures have long been called 'dorsal roots'. Spengel (1893) wished to express approval of and to use Bateson's term, but to avoid the impression of concurring in Bateson's homologies; so he simply used the name 'Wurzel'. Some of the Harrimaniidae have essentially similar strands, which have long been distinguished as 'dorsal crests'. In no species of the Spengeliidae has a root or crest been found to reach the epithelium, though several forms have short vestigial outgrowths of the collar nerve-cord.

The dorsal roots and the dorsal crests (or 'keels') differ chiefly in antero-posterior extent. The former structures are always short in this axis, as both types are in the transverse axis, so that a dorsal root is a round or nearly round strand of tissue, longest dorso-ventrally. The dorsal crest is long in the antero-posterior direction, usually one-quarter to three-quarters of the length of the collar. Furthermore, the crest is characteristically short dorso-ventrally, since the cord is close to the epithelium in these regions and species. There may be several roots, but usually only a single crest. The latter may, however, open to the epithelium several times, being for most of its length separated by the limiting membrane from direct continuity therewith. Except for these topographic features, dorsal roots and crests are essentially similar structures and will be treated together. The crests are structurally simpler, but there is no good evidence as to which is more primitive. In either case their nature is that of a dorsal extension of the tissue which forms the cellular mass of the collar cord medially. This extension pushes apart the laminae of the dorsal mesentery to reach the dorsal limiting membrane. Breaking through this, the cellular tissue of the root or crest becomes continuous with the outer epithelium. Instances occur, however, where a dorsal root is independent of the mesentery, for example, in front of its anterior margin. The opening in the limiting membrane of the dorsal epithelium by which direct continuity is established may be exceedingly small, often less than 10 μ in any direction in the crest of Saccoglossus pusillus. The root always contains nuclei, though what kind of cells these represent I am unable to say. Spengel (1893) felt sure they were not nerve-cells. Frequently, as in Saccoglossus, this is virtually all that makes up the structure, though a few nerve-fibres do pass through it as well (Spengel, loc. cit., to the contrary notwithstanding). But in many species the root has a well-defined external fibre layer and an internal cellular cord. The former condition is found in some roots and all crests; the latter represents the root at its highest development. Thus the fibre layer of the collar is clearly put into direct continuity with that of the outer epithelium through these roots.

Many dorsal roots exhibit either diverticula of collar-cord cavities or small, blind lacunae of their own. Successive roots in the same individual may possess such cavities or be entirely solid. Species with a continuous canal in the collar cord usually have only isolated lacunae in the roots, but may rarely have a continuous canal in the root opening into that of the cord. The fate of the cavities dorsally is naturally of interest. In the great majority of species the cavities do not enter the epithelium or open to the outside. An interesting exception has been reported by Willey (1899) and confirmed by Maser (1913). In Balanoglossus carnosus, and to a lesser degree in Balanoglossus numeensis, the dorsal-most cavity is continued into the epithelium as a horizontal, blind-ending, 'intra-epidermal canal' for distances up to 180 μ. Only a single instance has been reported of a dorsal root canal opening to the exterior. Van der Horst (1924) found one large specimen of Ptychodera bahamensis in whose dorsal root there was a continuous canal which communicated ventrally with the axial canal of the cord and dorsally with the outside.

Thus, histologically, the dorsal roots and crests are very simple structures. But they vary greatly between species in number, arrangement, topography, length, size, and constitution. There may be up to eighteen roots (Glossobalanus ruficollis), but most species have less than six; individuals having no roots but belonging to species which usually possess them have been encountered (Spengel, 1904a, Ptychodera flava funafutica). Roots may divide, anastomose, end short of the epithelium, or rejoin the nerve-cord. They have never been found paired. The variations between species will not be catalogued here; they are chiefly of taxonomic significance and may easily be found in the systematic literature or in Van der Horst's monograph (1927–39). Their morphologic significance and their role in the life of the individual worm are discussed below.

Trunk.—Regions of very different histologic character are included in this, the longest section of the body. The histologic character, furthermore, varies considerably from species to species. But with respect to the nervous system, over most of the surface of the trunk both these regional and specific differences are essentially quantitative. As over the entire surfaces of proboscis and collar, so for most of the trunk, the nervous system is essentially generalized and without local differentiations. Only in the mid-dorsal and the mid-ventral lines is this condition modified; in these positions there are concentrations of fibrous matter which constitute genuine cords. They will be separately described below.

The lateral plexus exhibits, in general, a gradient of development from anterior to posterior. Thus, the branchio-genital region of Saccoglossus pusillus has a nerve-fibre layer 4–7 μ deep, and a nerve-cell concentration averaging about 15 per 100 sq. μ of surface. These quantities decrease posteriad until, in the abdomen, between glandular ridges or islands, no visible nerve-fibre layer is present and no excess of nuclei over ciliary rootlet bundles can be detected. The glandular islands, besides exhibiting a sharp change in the staining affinities, a lengthening of cilia, and a general transformation in the character of the epithelium as compared with the surrounding non-glandular epithelium, show a well-defined nerve-fibre layer, 2–3 μ thick. These spots represent, in fact, the only places in the lateral epithelium where nervous tissue is demonstrable, in ordinary sections. A continuity between fibre layers of adjacent islands cannot be traced, though it is *a priori* probable that such exists. How tenuous it must be is evidenced by the appearance of the epithelium in interglandular areas. Simple, cuboidal, faintly ciliated cells, 5–10 μ in height, rest directly on the ever-prominent limiting membrane, showing no constricted 'cell feet' or any trace of punctate substance.

Local variations in degree of development of the nervous tissue occur over the surface of the trunk. Thus, over the gonads the epithelium may become thin and the nerve layer disappear; the superficial annulations of the integument may be reflected in thickenings of the nerve-fibre layer; or deep grooves, such as those between hepatic caeca, may lack that layer. Species like the ptychoderids with gill bars exposed have a distinct nerve layer on the outer side of the gills, whereas forms with well-developed branchial chambers and small, secondary gill-pores (Saccoglossus) have no visible nerve layer on the gills. In no species, apparently, do the anterior and posterior faces of the gills have visible nervous tissue. Again, the species of Ptychodera, as opposed to Saccoglossus, have no extensive non-glandular areas on the abdomen. The epithelium and its nerve layer are well developed right back to the anal end. Only in the grooves between hepatic caeca is a simple, cuboidal, non-nervous epithelium to be found.

The orientation of nerve-fibres in the lateral plexus of the trunk is nowhere restricted to any one direction. No predominance, certainly, of antero-posterior elements exists, though dorso-ventral fibres may sometimes appear in the majority. Some fibres pass in every direction, however, and no segregation could be demonstrated.

Nerve-cords.—Elongated concentrations of fibrous nerve tissue occur in the trunk along the mid-dorsal line, directly over the attachment of the dorsal mesentery, along the mid-ventral line, likewise marked by a mesentery, and in the transverse plane around the sides at the junction of trunk and collar. These three strands of thickened fibre layer will be spoken of as the dorsal (nerve) cord, the ventral (nerve) cord, and the circular connective, respectively.

The dorsal and ventral cords may be considered together for the sake of contrast. The dorsal cord (figs. 1, 2, 3, Pl. 2; figs. 4, 7, Pl. 3; fig. 9, Pl. 4) is represented by a thickening in the fibre layer of the epithelium in the mid-dorsal line. The increase in thickness of the fibre layer occurs at the expense of the superficial zones of the epithelium. In contrast, the mid-ventral epithelium as a whole is greatly thickened. The nerve-fibre layer makes up most of this increment in depth, but the nuclear zone also is usually thicker. Comparison of direct measurements of the two cords reveals a consistent difference in total cross-sectional area in favour of the ventral cord. The amount of this difference is not fully realized by a comparison of depths, for another highly characteristic distinction between dorsal and ventral nerve-cords is in the lateral extent of the fibrous thickening. The dorsal cord is always recognizable by its narrow, sharply delimited cross-section abruptly passing, laterally, into the general plexus, whereas the ventral cord is not only much wider, but its transition into the lateral plexus is often gradual. The ventral cord may, in fact, be actually shallower in certain regions than the dorsal, but its greater lateral extent always brings its total cross-sectional area to a figure higher than that of the dorsal cord. In one individual of Balanoglossus occidentalis a maximum depth of 110 μ for the dorsal cord, and only 80 μ for the ventral was recorded for a certain region; but the respective widths were such as to bring the total sectional area of the former to 29,000 sq. μ, of the latter to 37,000 sq. μ. Usually, however, the ventral cord is at least as deep as the dorsal, and the disproportion in area greater. Absolute figures mean little, because of the great variation between regions and species. For the sake of comparison with previous figures, however, we may mention typical measurements of Saccoglossus pusillus. The nerve-fibre layer of the dorsal cord in the branchial region may reach a depth of 50 μ, the sectional area amounting to 8,000 sq. μ, as against 75 μ and 18,000 sq. μ in the ventral cord in the same section. These figures diminish progressively posteriad until in the abdomen the depths may be no greater than 5 μ. Hess (1937), like others before him, reported that '. . . the dorsal (cord) is larger and better developed than the ventral'. But my own measurements of his species (Saccoglossus kowalevskii) agree entirely with those above in respect to a consistent preponderance in size of the ventral cord; whether the dorsal cord is 'better' developed or not, I will not argue; it certainly is differently developed from the ventral.

The fate of the cords in the terminal abdomen is interesting. In Ptychodera bahamensis, where a well-developed lateral plexus persists throughout the abdomen, the dorsal and ventral cords, a few micra anterior to the anus, are about 25 μ deep. Laterally, directly over the end of the abdomen the fibre layer is thickened to at least 20 μ deep. We may think of the cords here as extending right to the lips of the anus, there to bifurcate and meet around its sides. I was unable to detect, however, such an orientation of fibres as would suggest a circumanal ring. An interesting contrast is seen in Balanoglossus occidentalis in which the abdomen is differentiated into a thin-walled post-hepatic region and a conspicuously swollen terminal region. Further differences are seen in this species in the presence of a 'pygochord' (Willey, 1898), and in the character of the lining of the terminal intestine, which here is a deep, glandular epithelium possessing a very thin nerve-fibre layer, whereas that of Ptychodera is low, non-glandular, and without a visible nerve layer. It is perhaps surprising that in the more specialized terminal abdomen of Balanoglossus the dorsal cord should diminish until, several sections before the first breaking through of the anus in a transverse series, it is completely indistinguishable from the lateral plexus. The ventral cord persists somewhat longer, but there is no circumanal thickening by which the cords are continuous.

Differences between the dorsal and ventral nerve-cords extend to finer details as well. Over both cords the epithelial glands are significantly reduced in number, but this is more true of the dorsal than of the ventral. The radial fibres of indifferent epithelial cells are concentrated to each side of the greatest depth of fibrous matter in the dorsal cord, thus exaggerating its delimination from the lateral plexus. They frequently divide the peripheral-most nerve-fibres into conspicuous fascicles occupying alcoves between the radial elements but broadly continuous with the rest of the cord. The radial fibres of the ventral cord are equally well developed, but more numerous and more uniformly distributed. Reference has been made to the differences in number of nuclei of the two cords. Whereas in the ventral cord nuclei are considerably more numerous than in the adjacent lateral epithelium, in the dorsal cord of Saccoglossus pusillus the nuclei are less than half as numerous as in the immediately lateral epithelium. A typical figure for the branchial region is 10 nerve nuclei per 100 sq. μ of surface over the mid-dorsal line, 18 in a similar area parasagitally. In other species there may be, on the contrary, a concentration of nuclei in the dorsal cord, but not to exceed that of the ventral cord. The reduction in gland-cells and the proportional or absolute increase in the number of nuclei indicate that it is nerve-cells which are being concentrated in the cords. Corresponding with its greater number of nerve-cells and greater size, the ventral cord may differ from the dorsal with respect to fibre orientation. In both, the predominant direction is longitudinal, but the ventral cord may exhibit a greater number of diversely oriented fibres with but a central zone of longitudinal ones.

Although the dorsal and ventral nerve-cords are connected with each other throughout their extent by the lateral plexus, there occur in all species of enteropneusts thickenings of the nerve-fibre layer at the anterior end of the trunk where the ventral cord abruptly ends and the dorsal cord is continued into the collar cord. These thickenings extend through the lateral plexus from the ventral to the dorsal nerve-cord on each side. In some species they are poorly delimited. In no species are they very thick, both cords together probably never equal the cross-sectional area of the ventral cord at this level. In Saccoglossus pusillus, they rarely exceed 10 μ in depth. That they are important is attested by experiments (Bullock, 1940), and by the strikingly uniform orientation of their constituent fibres. No plexus exists here, but a well-developed conduction tract. The correlated tendency to an alinement of radial fibres is well seen here.

It is significant in relation to Hanström's theory (1928) of the origin of longitudinal nerve-cords, to be discussed below, that no indications whatever of lateral nerve-cords have been encountered in this study, nor have they been mentioned by earlier authors.

Epithelium of the gut.—A well-developed nerve-fibre layer exists in the lining of the buccal cavity. This is frequently thickened in the mid-dorsal and occasionally in the mid-ventral line. The figures for Saccoglossus pusillus are in the vicinity of 4–8 μ for these thickenings, 1–2 μ laterally. The buccal plexus is a direct continuation of the layer found over the outer surface of the collar. Van der Horst (1929) called the ventral thickening a 'pharyngeal nerve', but the term 'nerve' is probably better reserved for true internal bundles of fibres as opposed to medullated cords and thickenings of the epithelial plexus. The fibre layer can be followed back to the pharynx, where it disappears except in the epibranchial and hypobranchial strips. In some forms (Ptychodera) it may be detected in the pharyngeal face of the gills, but never on their anterior and posterior faces. Throughout the intestine the presence of nervous tissue is extremely dubious. In most regions nervous tissue is not visible; but in the terminal intestine of those forms which, like Balanoglossus, have a high, glandular epithelium, a very thin nerve-fibre layer is apparent.

The Internal Nervous System.—With the technics that have thus far succeeded in my hands I have found it impossible to identify with certainty as nervous any structure internal to the limiting membrane. There are, to be sure, many cells lining the coelom and scattered in it whose nature remains undetermined, and which, by their large nuclei, ample cytoplasm, and long, attenuated cell processes, could well be ganglion cells. But they are equally likely to be connective-tissue cells, peritoneal cells, or any of the strange and still unclassified types of mesodermal coelomic elements. A striking display of fibres in the coelom is seen in protargol preparations, such as impregnate selectively the nerve-fibres in the outer epithelium. Some of these may be followed across the limiting membrane into apparent continuity with fibres of the nerve layer of the epithelium. But that this elaborate plexus represents an internal nerve plexus, and the stellate cells whose processes contribute to it represent nerve-cells, is exceedingly dubious. Arguing against such an interpretation are an extensive and too elaborate plexiform appearance, considering the musculature to be innervated, a difference in staining qualities from known nerve-fibres, and a ubiquitous intercellular disposition in compact muscle layers. These argentophilic fibres are perhaps better regarded as largely the processes of parenchymatous or 'connective-tissue' cells for the present. However, it is possible that true nerve-fibres are present in this plexus, but have not yet been distinguished from its other elements.

Spengel (1893) found structures in one species, Glossobalanus sarniensis, which he regarded as an internal nervous system. Two pairs of delicate cords passing longitudinally in the angles of the dorsal blood-vessel appeared to consist of punctate tissue and the surrounding cells resembled nerve-cells. He could find no connexion with the internal organs or with the superficial nervous system.

In no other species of enteropneust has such a strand been identified. Concerning Glossobalanus elongatus, Spengel (1904*b*) specifically states that he could not confirm the existence of such a 'sympathetic' nerve. Nothing of the kind occurs in most of the species I have examined. In Balanoglossus occidentalis and Glossobalanus sp. (from La Jolla, California) structures suggestive of parts of Spengel's description have been seen. I am entirely uncertain whether the strands are indeed composed of nerve-fibres or that the adjacent cells are nerve-cells, but my observations do not extend or correct Spengel's description except in reporting the definite absence of such paravascular cords in most species examined.

The only other reference to internal nervous elements is that of Hess (1937). He reported a '. . . multipolar nerve network . . . in certain internal regions of the body . . .' in methylene blue preparations. This network is specifically mentioned only for the region of the proboscis near the heart. It is figured as a true anastomosing net whose cells are about 5 μ in diameter, multipolar, and protoplasmically continuous. 'From the ganglion cells . . . fibres, which are probably effectors, pass into the muscle fibres.'

It is impossible to deny the occurrence of nerve elements in the coelom, but it is significant that there are no layers, concentrations, ganglia, tracts, or nerves (with the possible exception of Spengel's paravascular cords), that the elements must be diffuse and few, and are probably confined to direct or terminal neurons or parts thereof, to the exclusion of any considerable numbers of intermediate or connecting neurons.

Discussion.

There must now be undertaken a brief statement of (1) the bearing of the present results on previous anatomical descriptions, (2) correlations with the available information on the physiology of the nervous system, and (3) certain evolutionary considerations in which this system is involved.

Anatomical Concepts.

Sense Organs.—The literature contains no convincing reports of sense organs in balanoglossids. Spengel's (1893) account of a 'sensory pit' in the proboscis of Stereobalanus canadensis does not suggest a sense organ,[1] nor do Van der Horst's (1930) figures of an organ, supposedly sensory, in the dorsal epithelium of Glossobalanus ruficollis. Assheton (1908) found a structure in the collar epithelium of Saccoglossus serpentinus which he supposed to be sensory, but he gave no histological details.

I have found on the base of the proboscis in several species (Saccoglossus pusillus, Saccoglossus kowalevskii, Glossobalanus minutus, and Ptychodera bahamensis, see fig. 17, Pl. 6) the pre-oral ciliary organ first described by Brambell and Cole (1939), and homologized with the wheel organ of Amphioxus and the hypophysis of Craniota. This organ consists of two very low parallel ridges in the transverse plane. The cilia borne by the cells which form the ridges are somewhat longer than those found elsewhere, and the rootlet cones stain more deeply. Contrary to Brambell and Cole's findings, the nerve-fibre layer appears to have no consistent thickening related to these ridges. The evidence does

[1] This is the only published species of the genus. In an undescribed species of Stereobalanus from Central California I find no such pit.

not justify the assumption of much greater sensitivity of the ridges than the adjacent epithelium. Rather the base of the proboscis as a whole, and not just the region of the pre-oral ciliary organ, is to be regarded as highly sensitive (Bullock, 1940).

It may be concluded that the Enteropneusta lack true sense organs. This is confirmed by the absence of any clear-cut case in the literature, and by the complete failure to find any evidence of such organs in this investigation.

Nerve-cell Types and the Constitution of the Epithelium.—Bateson (1886*a*) regarded '. . . the skin of Balanoglossus . . . as a collection of sensory cells ending in long fibres, which may either be connected to the central nervous system, probably by the longitudinal fibres of the "punktsubstanz" (i.e. those passing parallel to the plane of the epithelium, which we now regard as comprising the entire nerve layer) or may pass directly through this as motor-fibres into the muscles'. These motor-fibres are evidently Bateson's 'ciliated, tailed' epithelial cells which we recognize from his figures and description as the supporting fibres, or cell feet, of ordinary epithelial cells. Thus Bateson did not distinguish non-nervous, epithelial supporting cells and sensory nerve-cells, but conceived of the epithelium as consisting of (1) a very few ganglion cells (merely because 'of the antecedent probability of their occurrence'; he did not find them) and, (2) of ciliated, sensory cells many of whose processes passed directly into the muscles. That is, a reflex 'arc' of one neuron would be the rule. Our evidence to-day forces us to abandon many aspects of this view, although no evidence can be cited that would preclude the existence of one-neuron reflex 'arcs'. His concept of motor-fibres crossing the limiting membrane everywhere, diffusely, though very possibly correct in view of the evidence presented here, was an erroneous conception as he visualized it, and based on false histologic pictures. Spengel distinguished between the indifferent epithelial 'Stützzellen' and 'Nervenzellen'. The former he associated with the elongate dark-staining nuclei, the latter with the more basal, round or oval light-staining nuclei. Only when he noted a great discrepancy in numbers between the elongate nuclei and the 'fadenförmigen Zellenfüsse' (radial fibres) in favour of the former, in regions like the base of the proboscis, did he conclude that some of these nuclei represent sense-cells. He did 'not doubt that such cells occur also in other places', but evidently regarded them as less regular or numerous occupants of the epithelium than the 'Nervenzellen' or ganglion cells. Van der Horst believed that Spengel's conception of sensory elements corresponded to present-day 'true' or secondary sense-cells. The combined evidence from selective stains, epithelial counts, and the comparison of well-developed sensitive epithelia with simple non-glandular, non-nervous regions indicates that no secondary but only primary sense-cells occur in these organisms (as is true of all other invertebrates; see Hanström, 1928), that these cells are far more abundant than ganglion cells, and that in general they possess and account for the elongate nuclei. The ganglion cells do possess round or short oval nuclei, as Spengel supposed, but do not, as he thought, nearly account for these forms. I believe that in general the ciliated epithelial 'Stützzellen' have basal, round or short oval nuclei, as is especially evident in the simple epithelia where other cell-types are rare or absent. Spengel stated that elongate nuclei represent the indifferent epithelial cells even in the collar cord, but I have noted no such nuclei here, and a careful search through his plates shows that he figures none. The points mentioned, together with a cursory description of giant cells, are about the extent of Spengel's interest in the nervous system, aside from topographic anatomy of the cords.

Van der Horst (1927–39) goes no farther than to say that certain epidermal cells must, *a priori*, be sense-cells, and that he believes them to be primary sense-cells connecting with an asynaptic nerve set. Brambell and Cole (1939) describe and figure a type of cell in the epithelium which they believe to be a sensory nerve-cell. This is almost certainly a gland-cell.

Hess (1937, 1938) has offered the only picture of the cellular elements of the nervous system of an enteropneust supported by special nerve stains. His papers principally concern the existence and function of one cell type. This is a large, bipolar cell lying horizontally, that is, parallel to the plane of the nerve-fibre layer. It is described and figured from methylene blue and protargol stains of fragments of Saccoglossus kowalevskii. According to the scale on his figures, these cells are in general 27–36 μ long, a truly giant length for balanoglossids. Their processes may be very long, some being said to extend throughout the length of the proboscis, about 30 mm. They are usually darker at one end, which end contains the nucleus. None of the fibres figured branch. They are indicated as much larger than ordinary fibres of the nervous plexus. The entire cell is said to be in the nerve-fibre layer, and where fibres run in both directions, as in the proboscis, those oriented longitudinally are internal to those passing transversely. Fibres running obliquely are not mentioned or figured. The bipolar cells are described as least numerous on the ventral side of the trunk. specifically 543 cells per square millimetre, more abundant on the dorsal and dorso-lateral sides of the trunk, where 801 cells were counted per square millimetre, and thickest on the proboscis (no figures). In the trunk they are said to be oriented exclusively antero-posteriorly, in the general plexus as well as in the cords; and they are found as abundantly in the interglandular areas as under the glandular islands, dorsally, but only between these islands on the ventral side. The fibres are just as numerous under the islands as between them.

Now, this picture is a remarkable one. The results of the present study indicate that nuclei and cell-bodies are rare in the fibre layer, and that the plexus is characterized by fibres running in all directions, and unstratified. The plexus in the abdomen shows a conspicuous lack of longitudinal orientation, and experiments have shown (Bullock, 1940) the great difficulty of longitudinal conduction in the general plexus of the trunk, and the ease of transverse or circular conduction. Cells and fibres are said to be at least as abundant between glandular islands as under them, more abundant ventrally, whereas the nerve-fibre layer in the interglandular areas in sections is so thin as to be invisible, and no excess of nuclei over ciliary rootlet bundles occurs. The glandular islands, on the other hand, have a distinct fibre layer and a definite excess of nuclei over ciliated and gland-cells. Although considerable numbers of huge, basal, horizontal bipolar cells are described, no other types, especially vertical, primary sense-cells (bipolar, but with one process very short) are mentioned. (In fig. 2, 1938, a diagram of the epithelium, a single vertical 'sense-cell' is drawn, but no mention of such cells is made in the text; in fact, it is to be inferred that none exist.)

I do not doubt the existence of horizontal bipolar cells throughout the superficial plexus. I have seen and described such elements. But I have not encountered any such giant cells and fibres as those Hess represents, although such neurons should be easily found in ordinary sections without special stains. Further, I feel that the very regular distribution is doubtful. In my own methylene blue preparations appearances suggestive of uniformly longitudinal fibres were encountered in the trunk, but these were readily shown to be sub-epithelial muscle-fibres. (In this region there are no circular muscles, whereas in the proboscis there are outer circular and inner longitudinal muscles.)

But, most importantly, I question the representativeness of this concept. Hess apparently regards these bipolar cells as the only element, or at least the principal constituent, of the peripheral plexus (see quotations below). He does not recognize a division of nerve-cells in the general plexus into sensory, associative, and motor, although all three are indubitably present. The absence of the primary sense-cell (except for the single cell diagrammed) is remarkable considering its ubiquity in every other group from the coelenterates to the vertebrates. I do not believe that 1 per cent. of the fibres making up the nerve-fibre layer, even in the trunk where that layer is not very thick, could be accounted for by 801 cells per square millimetre. This means one neuron in a 35·4 μ square or in about 120 μ of a 10 μ section!

Finally, the interpretation which Hess has placed upon these cells seems open to question. He has assumed them to be the receptor organs of these animals, '. . . since the subepithelial (sic) nerve plexus of Dolichoglossus [Saccoglossus] is a bipolar plexus which is connected directly with the central nervous system, it is probably composed of sensory neurons which are concerned with the reception of stimuli and the conduction of impulses to the co-ordinating centre in the proboscis stalk and collar'. (Hess regards the collar cord and peduncle as a central nervous organ.) He specifically designated the horizontal bipolar cells as photoreceptors: 'Hence, because the elongated nerve-cell bodies described above are larger and more numerous in the more photosensitive regions, because they stained with the selective nerve stains used, and are the only cells that so stained that they could be the photoreceptor cells, because they lie at the base of the outer body epithelium and consequently resemble in their general location that of the photoreceptor cells in the earthworm, . . . and since they resemble in their morphology the retinal cells of vertebrates these elongate nerve-cell bodies have been designated the photoreceptors of Dolichoglossus.'

Since in all groups of animals with a nervous system, including the earthworm, the peripheral sensory cells (aside from secondary sense-cells, which are peculiar to vertebrates) are essentially vertical elements with a single nerve-fibre and a short distal process projecting towards the surface (see Hanström, 1928), it seems improbable that a horizontal cell lying in the basi-epithelial plexus and giving off a long nerve-fibre from each end is a sensory cell. It is more likely an intermediate or associative 'ganglion' cell.

Functional Correlations.

The general plexus of balanoglossids conducts diffusely and with apparent decrement, in the manner of a classical nerve-net (Bullock, 1940). Pieces of the body-wall show a high degree of autonomy, as is true with primitive net systems elsewhere. These properties are in agreement with and find their anatomical bases in the diffuse distribution of sensory cells, ganglion cells, interneuronal junctions, and motor innervation. But, whereas the nerve-net has generally been assumed to depend on anatomical continuity between neurons (see, for example, Parker, 1919), no evidence for such continuity was found in this study. The balance of evidence is in favour of discontinuity although this cannot be said to be demonstrated. These findings agree with other recent evidence (e.g. Bozler, 1927) to the effect that nerve-nets are not necessarily protoplasmically continuous. Especially significant in this connexion is the evidence of Pantin (1935 et seq.) that the property of 'decremental' conduction is probably an indication of a chain of neurons separated by synapses, each of which requires the 'facilitation' provided by two or more impulses to break down its resistance. The existence of diffuse conduction and the probability of synapses suggest, as the simplest explanation, that the synapses are unpolarized. Most textbook definitions of the synapse included the word polarized, but it is becoming questionable whether the property is necessarily or even actually true of the individual junction in higher animals. It may be instead an effective polarization due to numerical or anatomical relations. In any case, it is entirely conceivable that in primitive nervous systems the synapse is a potential barrier to conduction with no polarization, and that effective polarization developed gradually through various degrees as is seen to-day in many nerve-nets, for example, that of the balanoglossids.

The fact that the ventral nerve-cord is physiologically more important (less dispensable) than the dorsal agrees with the anatomical demonstration that the former is larger. It is to be noted also that most of the musculature of the trunk is ventral. Both lines of evidence have emphasized that the cords, including the collar cord, are probably chiefly conducting tracts and only to a lesser extent ganglionic. Evidence from both approaches indicates that this is truer of the dorsal cord of the trunk than of the ventral. The importance of the circular connectives, despite the continuous communication of dorsal and ventral cords through the general plexus, was shown by the considerable effect of cutting these connectives.

The anatomical picture in general is that of a very primitive and simply organized nervous system, with a small ratio of associative or connective neurons to sensory neurons. The functional picture agrees with the anatomical, being characterized by simple, undifferentiated and largely local responses.

These considerations bring up the historically interesting question of whether the collar cord, or that cord together with some or all of the other nervous concentrations, should be regarded as a central nervous system. It seems clear now that in so far as a central nervous system is a region involved in all ordinary reflexes, which contains all the intermediate neurons, and to which pass all the sensory nerve-fibres, the cords of balanoglossids are not a central nervous system. Only in so far as they represent specialized conduction tracts and may have a modifying effect on such reflexes as do traverse them are they 'central' organs. Their organization and dispensability emphasize the gulf which separates them from the central nervous systems not only of vertebrates but of most invertebrates. In view of the generally accepted connotations of the term, I prefer to regard the enteropneusts as lacking a central nervous system. Spengel (1893) denied the collar cord the status of a central nervous system, but on morphologic not neurologic grounds. Hess (1937, 1938) speaks of the central nervous system of these animals, and goes so far as to call the peduncle a 'centre' (for light reception). I do not believe the facts available justify the assumption of 'centres' in the ordinary neurologic meaning of that term.

Phylogenetic Speculations.

A number of problems of morphologic interpretation demand at least cursory treatment. One of these arises from the existence of either a continuous canal or blind lacunae in the collar cord. These structures have for long challenged speculation and it may be said of them, as of most others of the morphological features prominent in phylogenetic discussions, that the authors of forty years ago were in as favourable a position for their interpretation as we are to-day. The essential facts became known when Spengel published his monograph (1893), and no additions or alterations significantly changing their totality for morphological purposes have been made since. The problem, therefore, is simply one of evaluating the facts and applying changing points of view. The present paper proposes no original phylogenetic argument. My objective in this connexion is to make available a summary statement of the data and of the principal homologies that have been suggested relating to the nervous system.

The general facts of the comparative anatomy of cavities in the collar cord have been presented above in their place. A few unusual cases, considered by their describers to be significant, may be mentioned here. And the known embryological facts, incomplete as they are, should be indicated. Bateson (1886*a*) described an anterior axial canal which reached only part way into the cord in Saccoglossus kowalevskii, and which was followed by blind cavities of the kind we have called lacunae. He wrote: 'It is possible that the spaces [lacunae] . . . may in some indirect manner communicate with the neural tube [anterior canal].' But Spengel (1893) denied this possibility, and every subsequent worker has furthered the evidence against any such intercommunication. My observation on Saccoglossus kowalevskii and other species have also failed to confirm Bateson's belief in any way. Spengel (1893) mentions some instructive cases of anomalous individuals. A specimen of Glandiceps talaboti, a species which normally has scattered lacunae only, was encountered whose collar cord was traversed by a continuous axial canal through at least the anterior half. Schizocardium brasiliense likewise usually exhibits isolated cavities only; but one animal possessed not only lacunae (lateral, as is usual) but a median axial canal. Finally, young individuals of Glossobalanus minutus exhibited axial canals, quite unlike older members of the species. Later (1904*b*) Spengel described a species, Glossobalanus elongatus, whose cavities he regarded as an interrupted axial canal. In several species lacunae are present in the dorsal nervous concentration of the peduncle. All these cases he considered as evidence that the 'Axencanal' and the 'Markhöhlen' are not to be confused and that they had, if not a different, at least an independent origin.

The development of the parts in question should offer most illuminating clues, but, unfortunately, the appropriate stages have been described for only three species, and only incompletely. Bateson (1884, 1885) describes the collar nerve-cord of Saccoglossus kowalevskii as delaminated from the medial dorsal ectoderm and at first solid. The description of cavities goes no farther than to say that at the anterior end of the collar, by simultaneous forward growth of the organ and backward (horizontal) invagination at the point of junction of the internal nerve-cord with the superficial nervous layer of the peduncle, a lumen is formed which extends a 'short distance into the cord'. Bateson's stages do not reveal whether the definitive lacunae arise from this lumen by a process of tissue invasion, which would not account for their occurrence throughout the cord, or de novo in the solid rod of nervous tissue, which would seem unlikely in view of their epithelial character and ciliary apparatus. The situation is simpler in the other two forms, both having indirect development by means of Tornaria larvae (whereas Saccoglossus kowalevskii develops 'directly', without such a larva). In the Tornaria of New England Morgan (1891) showed a modified form of invagination. The median dorsal plate sinks bodily below the general level and the edges roll over it, finally meeting and fusing to leave a cord, solid in some regions, provided with a lumen in others—whether clearly an enclosed bit of outside space or not is uncertain. Again, the numerous definitive lacunae (for this species, probably Balanoglossus aurantiacus, has many isolated lacunae in the adult) cannot be exactly related to the one, or few, embryonic cavities, in the present state of our knowledge. In Tornaria morgani, considered by Stiasny-Wijnhoff and Stiasny (1927) to be the larva of Ptychodera bahamensis, Morgan (1894) demonstrated a simple invagination producing a continuous lumen, from which doubtless is derived the continuous axial canal of the adult Ptychodera. We may say, therefore, that certainly in some species, and possibly in others, the cavities of the collar nerve-cord represent portions of outside space, either enclosed or still continuous therewith. This statement, however, must be considered as extremely tentative, based as it is on but one clear and two incomplete accounts of the embryology of the Enteropneusta. The scattered lacunae of the majority of species are not embryologically obvious derivatives of a continuous axial canal by fragmentation or tissue invasion, and no transitions between the two conditions are known. Nevertheless, the latter is commonly regarded as the primitive enteropneust condition and the former as derived from it.

Similar difficulties attend the interpretation of the dorsal unpaired strands which connect the collar cord and the outer epithelium. From the taxonomic distribution we may regard the possession of a dorsal root or crest as the primitive condition. The significance of these structures for the individual can only be conjectured. They certainly make possible an exchange of fibres between cord and integument; but the fewness of these fibres in most roots and all crests, and the complete absence of these structures in all spengeliids and in occasional members of species which normally have them, argue for their very slight functional importance. Their cells, furthermore, do not appear to be nerve-cells. The morphologic significance of these dorsal, unpaired connecting strands is even more difficult to ascertain. Their embryology is not known. The simplest assumption would seem to be that they represent rests of the embryonic connexion with the epithelium which exists before delamination or invagination of the collar cord. Spengel believed them to be rests left by the anterior and posterior margins of the collar as these grew forward and backward, respectively.

Regarding homologies it is difficult to make general conclusions without considering simultaneously the morphologic features of all the other organ systems. This is beyond the scope of this paper. But it is instructive to examine the specific homologies that have been applied to the nervous system, apart from others, because that system has represented one of the classical arguments for the most important phylogenetic theory relating to enteropneusts. This theory identifies the enteropneusts with the chordates, as is well known. It is, to my knowledge, the only important positive suggestion of the relationships of the balanoglossids that has been advanced in fifty years. Moreover, earlier alternative theories either lost tenability with increasing knowledge of the animals concerned (Agassiz, 1873; Eschscholtz, 1825) or never gained any considerable support (Spengel, 1893). The obvious and frequently proposed homology with respect to the nervous system relates the collar cord of enteropneusts with the neural tube of chordates. The basis for this homology is the dorsal position and hollow character of the collar cord. The former feature depends very largely for its significance on the fact that in this particular region of the body no ventral cord exists, whereas in the greater part of the body a ventral as well as a dorsal cord occurs and is indeed larger and more important. The hollowness of the collar cord is pronounced in only a few species; in most it is only suggested; but it may well represent the primitive condition. Koehler (1886) has compared the tissue which interrupts the continuity of the axial canal to those grey commissures of the vertebrate brain which cross the ventricles, but it is still doubtful whether the discrete lacunae of balanoglossids arise by invasion of a once continuous cavity. Hanström (1928) has voiced the obvious corollary of the general homology of axial canal and neural tube by comparing the indifferent epithelial cells which line the former with the ependymal cells which line the latter. It is plain that if one homologizes the collar cord and the neural tube he must be willing to limit the correspondence to the anterior end of the latter tube and to accept the superficial, mid-dorsal nerve-cord of the trunk as corresponding with the rest of the vertebrate neural tube. He must further, of course, hypothecate a complete loss of the ventral cord and circular connective. Willey (1899) not only did this, specifically delimiting the extent of neural tube anterior to the trunk, but went farther and found the unfused medullary folds of the trunk:

'The medullary tube of the collar of Enteropneusta is the homologue of the cerebral vesicle only of Amphioxus and of the Ascidian tadpole and probably represents no more than the primary fore-brain (thalamencephalon)[sic] of Craniota. . . . Just as the medullary tube of the collar is admittedly an invaginated portion of the dorsal nerve-trunk, so the medullary folds which arise and fuse to form the medullary tube are to be regarded as specializations of the anterior portion of pleural folds which are retained in the Ptychoderidae as the genital pleura.' (Italics Willey's.)

These genital pleurae are great, wing-like folds of the body-wall of ptychoderids, ventral to the gill-slits and occupied by gonads. In the same connexion, he states farther on: '. . . the genital folds of Enteropneusta, the atrial folds of Amphioxus and the medullary folds of Vertebrata belong to the [same] system of pleural folds of the body-wall, and are differentiations from a common primordium.' (Italics Willey's.)

Assuredly, then, if phylogenetic precursors of the vertebrate neural tube are so clearly represented in the balanoglossid, the dorsal roots must have some profound significance. Bateson (1886*b*) found their homologues in the paired dorsal roots of vertebrates, and Willey (1899) in the epiphysis of the Craniata! Hess (1938) carries the detailed homology still farther, comparing his bipolar 'photoreceptor' cells with rods and cones of the vertebrate eye; some resemble rods, some cones. And, according to him, all 'are chordate-like in being embedded in the tissue of the central nervous system as in Amphioxus', though they represent an earlier stage in that they are also found throughout the general plexus. It would seem doubtful how characteristic of chordates are sense-cells which are in their most primitive position—scattered throughout the integumentary epithelium. These topographical relations are true of both Hess's bipolar cells and the probable actual photoreceptors, the undifferentiated primary sense-cells. But it is, unfortunately, not true that these latter occur in the collar cord which was most of Hess's 'central nervous system'. (He included the proboscis stalk, wherein photoreceptors do occur.)

All these corollary homologies stem from, and probably depend on, the primary comparison of the cavities of the internal portion of the enteropneusts' dorsal cord and the cavity of the chordate central nervous system. If it were not for this hollowness it is very doubtful how vigorously these homologies would be pursued. And yet, the existence of a roofed-over nerve-cord, or of invaginated, even hollow 'ganglia', is not new with the chordates. Hanström (1928) has reminded us of the cerebral ganglion of rotifers like Asplanchna which is formed by invagination, and of the cavity in the cerebral ganglia of many ectoproct bryozoans which persists in the adult. Finally, I should like to call attention to the cross-section of the arm of ophiuroid echinoderms. The relations are very like those in the collar cord of enteropneusts—an internal tube with the nervous tissue concentrated on the inner side, cellular portion nearest the cavity, and fibre matter on the periphery. These instances may mean nothing for the present problem, but are mentioned to suggest that the mere existence of cavities in a portion of the nervous system should not be taken as prima facie evidence of a chordate-like central nervous tube.

The argument for chordate affinities from the condition of the nervous system I regard as weak, considered by itself. But it is important to say that the general concept of the nervous system of enteropneusts to which we are brought, emphasizing as it does the primitive level of organization achieved, does not argue at all against the theory of chordate affinities. And the reservations made above as to the classical morphological homologies do not in the least add up to negative arguments. My own position is one of concurrence in the thesis of chordate affinities, based primarily on other grounds than that of the nervous system. Of these the chief, to my mind, are the series of embryological characters in which the two groups agree. 'Chordate affinities' does not necessarily mean inclusion in the phylum Chordata. I am not prepared to deliver an opinion on this question depending as it does not only on the morphologic facts but on the concept of the phylum as well.

Homologies with lower groups are even more difficult. The problem essentially reduces itself to the question of what bases can be found for identifying the nerve-cords of the present group with those of a second group, but not of a third. Or, must we place the nervous system in a position unique among the organ systems by declaring homologous the principal nerve-cords of all animals? No definite suggestions of the former type are known to me, involving Enteropneusta. But the latter alternative is just what Hanström (1928) has taken in his orthogon theory. All the principal longitudinal nerve-cords are derived, according to this theory, from an original series of, probably, eight paired longitudinal condensations of the diffuse plexus of coelenterates, connected by commissures. Such an orthogonal pattern is still found in ctenophores and certain platyhelminths. Further concentration of the nervous system has resulted in the fusion, loss, or hypertrophy of the members of the original orthogon. Commissures for the most part cannot be traced phylogenetically, but Hanström believes the longitudinal cords of most groups can be. Thus the two median cords of balanoglossids are supposed on this view to represent fusions in each case of two lateral cords, although no evidence remains of an originally paired condition. This group would resemble in this feature the nematodes (differing, however, in many other ways). But the dorsal cord and the ventral cord would separately be comparable directly to all median dorsal and median ventral or dorso-lateral paired and ventro-lateral paired cords, respectively, throughout the animal kingdom. They, moreover, would be homologous with all longitudinal cords as derivatives of the same original series. Many groups show traces of other members of the orthogon than those principally developed in that group. Thus the nematodes have small lateral cords in addition to the median dorsal and ventral cords. I have failed to discover in the balanoglossids any trace of lateral cords, and no previous author mentions them.

A comparison of the nervous system of enteropneusts with that of other invertebrates on the basis of the level of organization or the complexity achieved, quite apart from phylogenetic identification, is significant. The present group is immediately seen to be far below all those with internal ganglionic systems. Detailed consideration of the degree of histologic elaboration and physiologic complexity as we now see it shows it to be on the same general level as the lowest of those with diffuse, superficial, aganglionic systems. Specifically, the balanoglossid does not show nearly the degree of nervous organization found in the annelid, nematode, nemertean, platyhelminth, or echinoderm and is more closely comparable in this respect to the ctenophores and coelenterates, though it lacks the sensory discrimination and diversity of response even of some members of these groups.

SUMMARY.

1. Results of a detailed study of the nervous system of Saccoglossus pusillus, with comparative material of about two dozen other species of Enteropneusta, are presented.

2. The primary feature of the enteropneust nervous system is its position within the superficial epithelium. Pertinent relations with non-nervous elements of the epithelium are described. The indifferent, ciliated cells elaborate supporting fibres in those regions where the epithelium is well developed and nervous tissue is concentrated. Such cells are considered to represent neuroglia in its most primitive form. The fibres appear in places to be continuous with the ciliary rootlet cones.

3. Nerve-cells are distributed diffusely in all the epithelia of the body, with certain exceptions such as the intestine, gills, coelomoducts, and the non-glandular areas of the abdomen of some forms. Both sensory and connecting and possibly also motor neurons occur here; but the sensory cells greatly predominate, often outnumbering all other epithelial cell-types combined. However, but one morphologic type of sensory cell—a true primary sense-cell—and no sense organs seem to be present. The thesis of Hanström is borne out that the low order of complexity of the nervous system as a whole is correlated with a low order of development of sensory structures. This in turn is correlated with a sluggish bottom living habit of life.

4. The nervous tissue is shown to be conspicuously undifferentiated. All nerve-cell processes are alike and resemble the most primitive nerve-fibres. A single exception is formed by the giant nerve-cell fibres, of which a few dozen exist in the nerve-cords. The absence of strata, tracts, and special neuropile-like regions as well as of elaborate nerve endings, ganglia, nerves, and 'nuclei', is impressive. Following the neurologic principle that complexity of function is reflected in complexity of structure, this is taken to mean a low degree of functional specialization.

5. Indications of several kinds agree in suggesting that the relations between neurons are something other than proto-

plasmic continuity. In the sense that nerve-fibres from different neurons are discontinuous the enteropneust nervous system is tentatively to be regarded as synaptic. Experimentally, however, the plexus has been shown to function as a nerve-net. It is proposed that such physiologic behaviour be taken to indicate a net in the sense of diffuse conduction, but that it does not predicate anatomical continuity of the fibres of the net. Such a picture requires the assumption of unpolarized synapses and the facts derived from the present organisms are taken to be evidence for this assumption.

6. Other primitive characters are described. The synapses are unlocalized, being scattered throughout the plexus. No special structural modifications have been developed at the synaptic endings. Connexions with the interior across the limiting membrane, heretofore unknown, are astonishingly difficult to demonstrate, but they must be assumed to exist and evidence is accumulated that they are diffuse. The widely scattered sense-cells, synapses, ganglion cells, and connexions with the interior are correlated with, and account for, the experimentally demonstrated autonomy of small pieces of the body-wall.

7. The general plexus is locally thickened and modified (1) in the cords of the mid-dorsal and mid-ventral lines of the trunk, (2) circularly around the junction of the collar and trunk, (3) through the dorsal collar coelom as an internal, primitively hollow, medullary strand, and (4) on the dorsal side of the peduncle. These are primarily conduction paths and are only secondarily important as ganglionic or modifying regions. The ventral cord in the trunk is shown to be larger and more important than the dorsal. In the sense of an organ which is involved in all reflexes, which contains all the intermediate neurons, and to which pass all sensory nerve-fibres, the balanoglossid has no central nervous system.

8. Internal to the limiting membrane no concentrations of nervous tissue are known with certainty to occur. No nerves, ganglia, or layers have been developed. As yet inadequately demonstrated, the internal nervous sytem can at most be only a sparse and diffuse system of cells and fibres communicating

across the limiting membrane with the superficial plexus, at the least nothing but motor axons passing from cell-bodies in the integument inwards to the muscles.

9. The histologic evidence supports the previously demonstrated physiologic picture placing the Hemichordata in respect to the level of complexity of the nervous system below all other groups of animals with nervous systems except the coelenterates and ctenophores. No evidence is adduced that this primitiveness is secondary rather than original. In numerous histologic respects the enteropneust nervous system resembles that of Echinodermata and Phoronidea, but is simpler than either.

10. The chordate affinities of the balanoglossids are here accepted. But the strength of the argument from the nervous system is considered to have been overdrawn. No aspect of the general picture of primitiveness now demonstrated is, however, considered to argue against these affinities.

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EXPLANATION OF PLATES

PLATE 2.

Semi-diagrammatic representation of an enteropneust of the genus Balanoglossus.

Fig. 1.—Anterior end extending into the branchio-genital region.

Fig. 2.—Posterior end of genital region and transition to hepatic region.

Fig. 3.—Post-hepatic abdomen. This is swollen, as shown, in some species, but in many it tapers and exhibits glandular islands separated by non-glandular epithelium.

ar, abdominal region; *b*, buccal cavity; *bc*, branchial chamber; *bd*, 'Branchialdarm', upper portion of pharynx; *c*, collar; *cc*, coelom of collar; *cnc*, collar nerve-cord; *cv*, cardiac vesicle; *dnc*, dorsal nerve-cord; *dr*, dorsal root; *dv*, dorsal vessel; *g*, gonads; *gl*, glomerulus; *go*, genital opening; *gp*, gill-pore; *gr*, genital ridge; *gs*, gill slit; *gw*, genital wing (pleura or ala); *h*, heart; *hc*, hepatic coecum; *hr*, hepatic region; *lm*, longitudinal musculature of trunk; *m*, mouth; *nd*, 'Nahrungsdarm', ventral portion of pharynx; *ns*, nuchal skeleton; *p*, proboscis; *pc*, perihaemal coelomic pouch of trunk coelom; *pm*, proboscis musculature; *pp*, proboscis pore (a coelomoduct); *s*, stomochord ('notochord') with cavity opening into buccal cavity; *t*, trunk; *tc*, coelom of trunk; *vnc*, ventral nerve-cord; *wi*, wall of intestine.

PLATE 3.

Photomicrographs of sections prepared by the Bouin's-nitrocellulose-Masson technique.

Fig. 4.—Mid-sagittal section through anterior end of trunk, collar, and base of proboscis of Saccoglossus pusillus, a species without continuous axial canal. Dorsal to the right, anterior end up. × 33.

Fig. 5.—Dorsal part of similar section of Ptychodera bahamensis, a species with continuous axial canal (interrupted in this section near the posterior neuropore by a bend of the body which makes the lower part of the figure slightly parasagittal). Dorsal to the left, anterior end up. × 30.

Fig. 5*a* on overleaf, mid-sagittal section of anterior end of Ptychodera showing portion drawn in Fig. 5.

Fig. 6.—Transverse section through the collar of Balanoglossus occidentalis. × 12.

Fig. 7.—Transverse section through the anterior (branchiogenital) region of the trunk of Balanoglossus occidentalis. × 12.

PLATE 4.

Figs. 8, 9, 10.—Bouin's-nitrocellulose-Masson; fig. 11, Zenker-nitrocellulose-Koneff.

Fig. 8.—The ventral nerve-cord in a transverse section of the branchiogenital region of Balanoglossus occidentalis. Dorsal to the right. × 114.

Fig. 9.—The dorsal nerve-cord in the same section. × 114.

Fig. 10.—The dorsal portion of the peduncle of the same animal in transverse section. × 110. Fig. 10*a* on overleaf, transverse section of peduncle.

Fig. 11.—Dorsal portion of transverse section through the proboscis of Saccoglossus pusillus, showing the dorsal cord of that region. The proboscis complex is also shown. Dorsal upwards. × 130.

PLATE 5.

Figs. 12, 13.—Bouin's-nitrocellulose-Masson; fig. 14, Flemming's strong with acetic-nitrocellulose-Masson.

Fig. 12.—The collar nerve-cord in a transverse section of Balanoglossus occidentalis. The dorsal mesentery of the collar coelom, with small blood-vessels separating its two lamellae, is attached asymmetrically in this section. The great dorsal vessel is just ventral to the nerve-cord. Longitudinal muscles occupying the perihaemal extensions of the trunk coelom are seen at right and left. A giant nerve-cell fibre is emerging from the cellular portion of the cord at the left to run medially in the fibre layer. The lacunae are distinguished from other white spaces in the cellular portion (glands) by their dark-stained lining (basal granules) and the cytoplasmic fibres radiating from them. ×150.

Fig. 13.—A dorsal root in a similar section of the same animal at another level. The section passes through the centre (antero-posteriorly) of the root only ventrally, near the nerve-cord. Here it can be seen that most of the root consists of 'cellular' material, only a thin nerve-fibre layer occupies the periphery. Dorsally the root communicates with the superficial epithelium. Many small and only virtual lacunae can be seen in the cellular region. ×80.

Fig. 14.—Giant nerve-cell in the collar cord of Saccoglossus pusillus, transverse section. The single process dichotomizes and the two fibres pass into the fibre layer. A dorsal 'crest' is also shown connecting the cord and the overlying epithelium. Flemmings' strong with acetic-Masson. ×570.

Plate 6.

All figs.—Bouin's-nitrocellulose-Masson.

Fig. 15.—Transverse section of the body-wall in the floor of the hepatic region of Ptychodera bahamensis. The outer epithelium above, the gut below. The trunk coelom is occupied by muscle-fibres. Ciliary rootlet cones are suggested in outer epithelium. The zones of the epithelium, the segregation of nuclei, the supporting fibres and, in the gut epithelium, the absence of a visible nerve layer are shown. ×400.

Fig. 16.—An oblique section through the proboscis epithelium of Ptychodera bahamensis to show how the limiting membrane loses the appearances of a sharp boundary. The short, heavy, dark-staining fibres immediately inside the limiting membrane are the circular muscles. ×450.

Fig. 17.—Mid-ventral epithelium at the base of the proboscis, in sagittal section, Ptychodera bahamensis. Anterior to the left. Note the sudden transition from a highly nervous epithelium to the left (extends beyond the margin of the figure for some distance, slowly thinning to the general average of the proboscis epithelium) to a glandular and less nervous epithelium to the right. The same region is shown at lower magnification in the upper right corner of fig. 5. The horizontal fibre layer at the right and the sharp limiting membrane separating the nerve-fibre layer from the coelomic lining are well seen. The figure also shows the 'pre-oral ciliary organ' of Brambell and Cole, represented by the region of longer cilia. ×415.

PLATE 7.

All figs.—Susa-nitrocellulose-protargol.

Fig. 18.—Portion of the peduncular nervous concentration of Saccoglossus pusillus stained for nerve fibres. The surface of the epithelium is at the right. Note sensory fibres leaving the nuclear zone to enter the plexus, and the negative images of radial supporting fibres. ×750.

Fig. 19.—The same at higher magnification. Epithelial surface up. ×2,000.

Fig. 20.—The nerve-fibre layer in the proboscis epithelium. Nuclei of epithelium above; below, the proboscis coelom occupied by muscle-fibres (dark-staining bodies about the size of nuclei) and an argentophilic network of uncertain nature. The limiting membrane is unstained but lies immediately below the nerve-fibre plexus. In this field of view could be seen numerous thornlike nerve-fibres apparently penetrating the limiting membrane, but they cannot be traced with certainty to the muscles or into the argentophilic network of the coelom. This is the only anatomical evidence for communication between the nervous system on one side of the limiting membrane and the muscles on the other. The limitation imposed by the narrow focal plane of the photograph is particularly acute in this situation. A swollen goblet gland in the epithelium. ×2,000.

Fig. 21.—Another and more typical field of view in the same section, showing no apparent crossing of the limiting membrane. ×2,000.

PLATE 8.

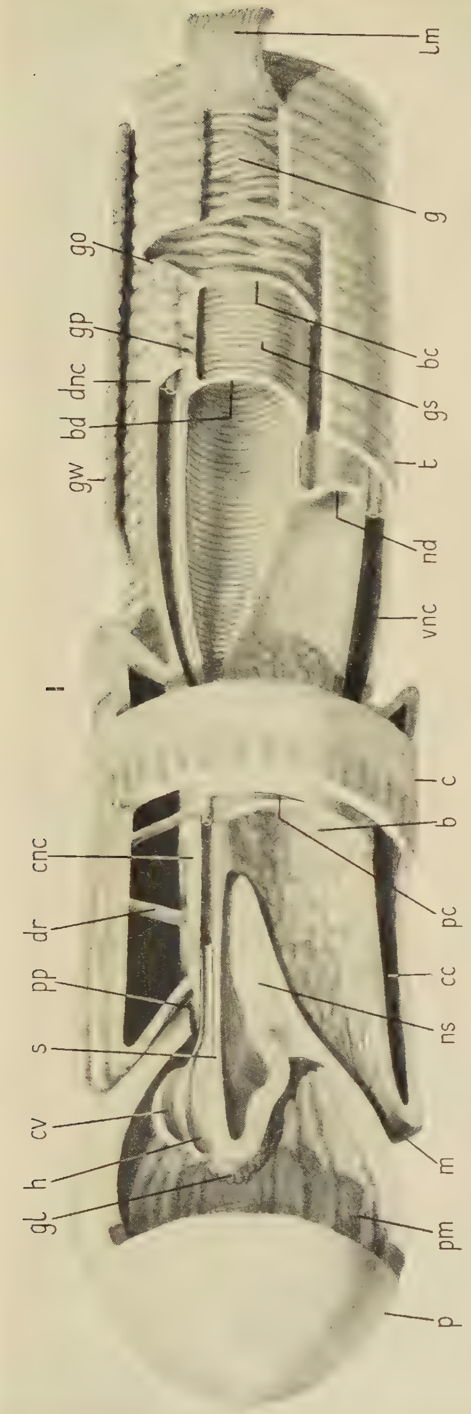
Figs. 22–31.—Sketched from still living specimens of Saccoglossus pusillus stained with methylene blue. All *ca.* ×1,500.

Figs. 22–5.—Primary sense-cells which stand perpendicular to the surface of the epithelium and reach or nearly reach that surface. The thick distal process may be two or three times as long as shown or apparently absent (fig. 24).

Figs. 26 and 27.—Indifferent ciliated epithelial cells which are occasionally stained. Note multiple and branched end feet, corresponding to the radial supporting fibres.

Figs. 28–30.—Various sorts of bipolar neurons seen deep in the epithelium parallel to its surface. Note intracellular fibrillae.

Fig. 31.—A multipolar neuron, likewise found at the base of the epithelium. Note that all processes are alike and are not dendritic.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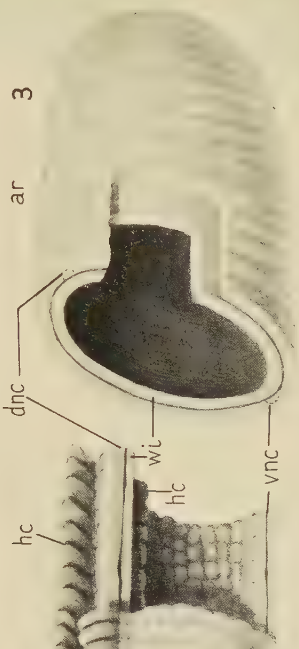
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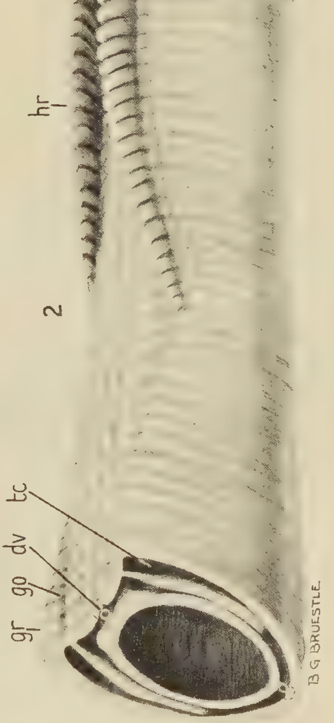
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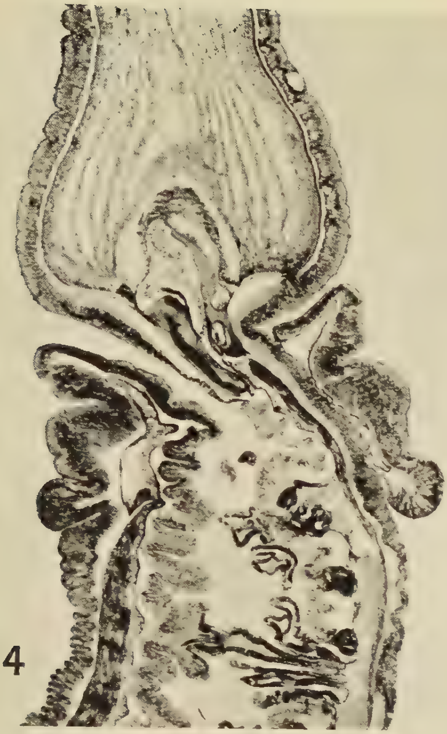

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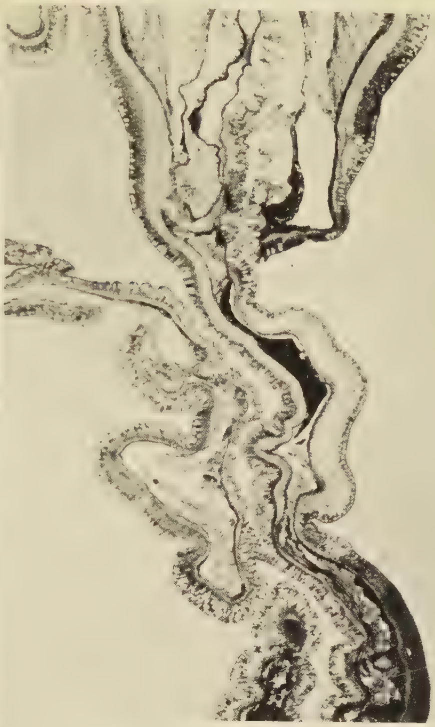

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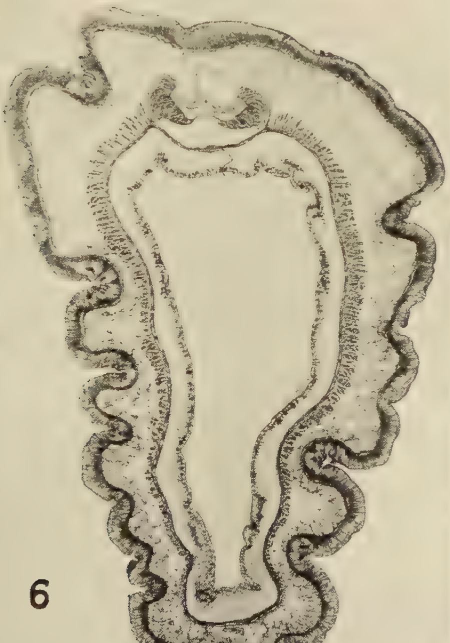

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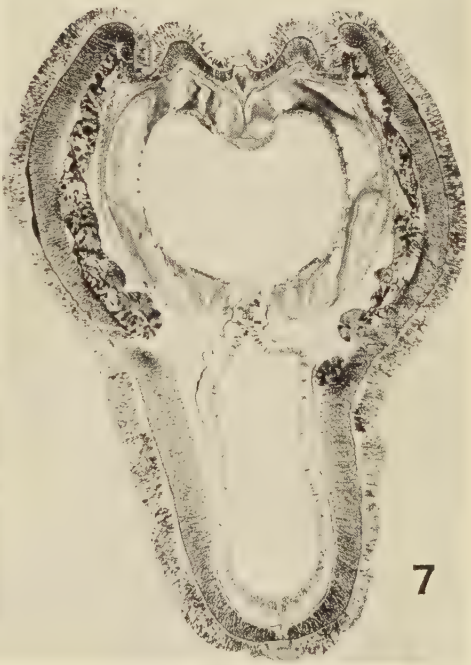

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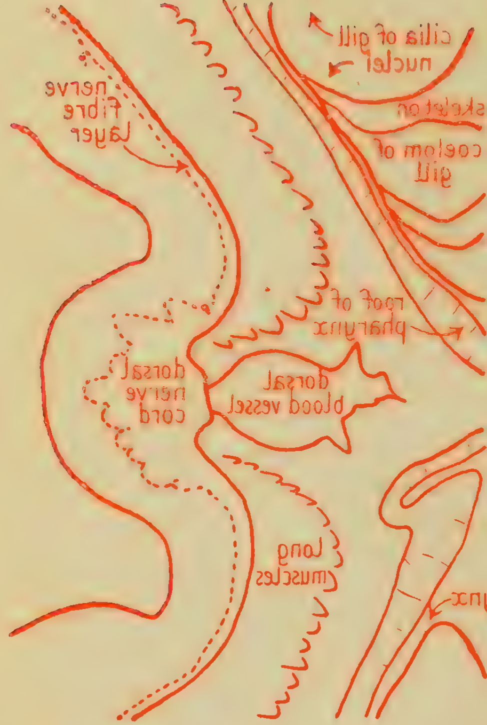
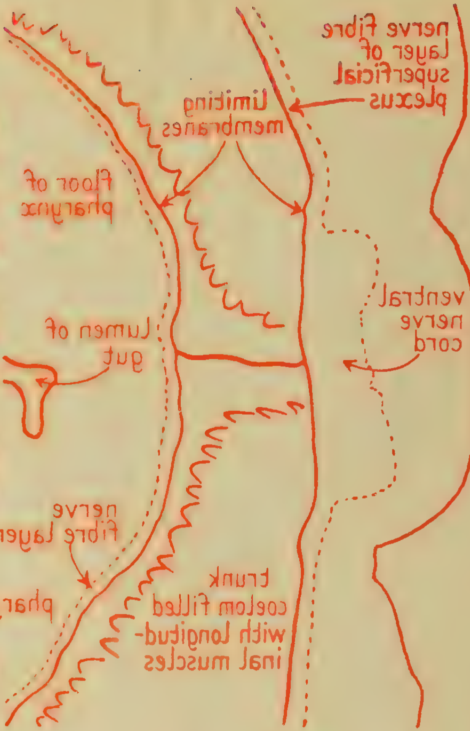
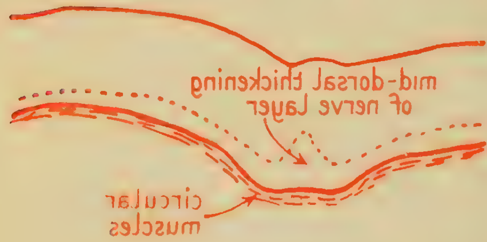
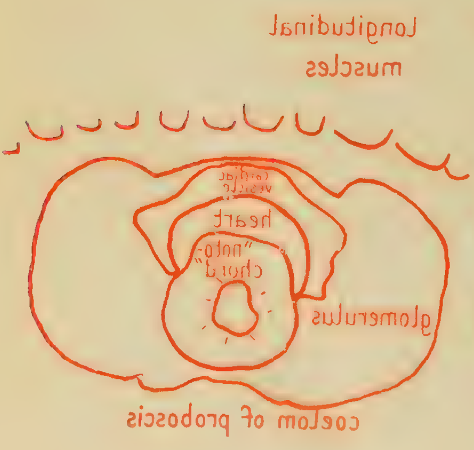
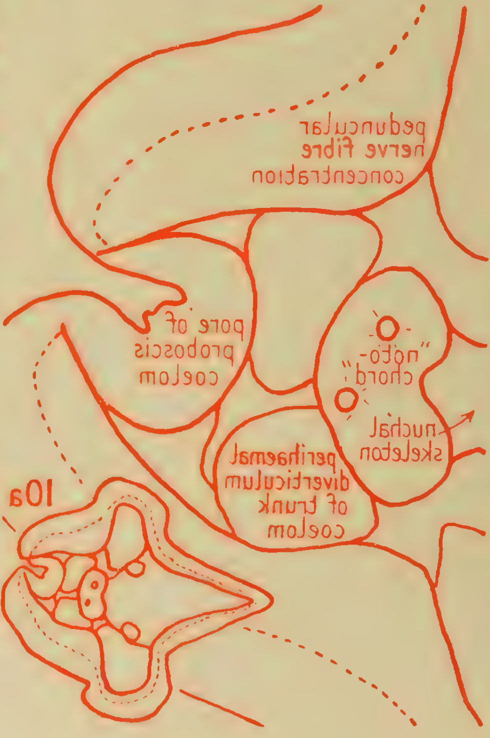

cilia of gill
nuclei
skeleton
coelom of gill
nerve fibre layer
roof of pharynx
dorsal nerve cord
dorsal blood vessel
long muscles
pharynx

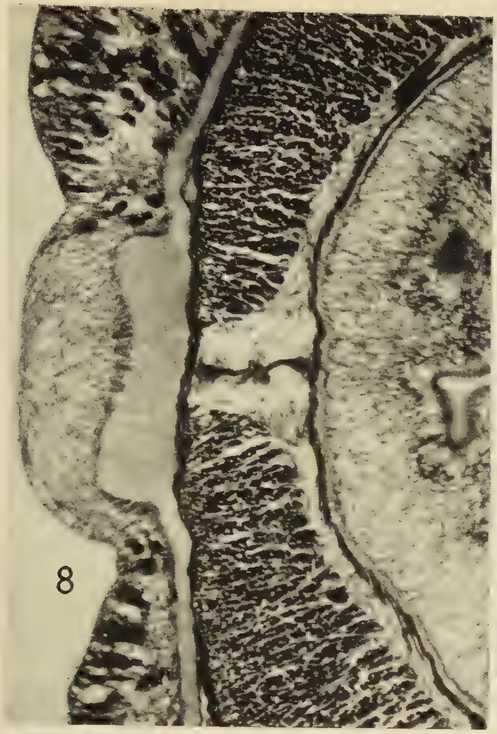

nerve fibre layer of superficial plexus
limiting membranes
floor of pharynx
ventral nerve cord
lumen of gut
nerve fibre layer
trunk coelom filled with longitudinal muscles


mid-dorsal thickening of nerve layer
circular muscles


longitudinal muscles
cardiac vesicle
heart
"noto-chord"
glomerulus
coelom of proboscis

10a


peduncular nerve fibre concentration
pore of proboscis coelom
"noto-chord"
nuchal skeleton
perihaemal diverticulum of trunk coelom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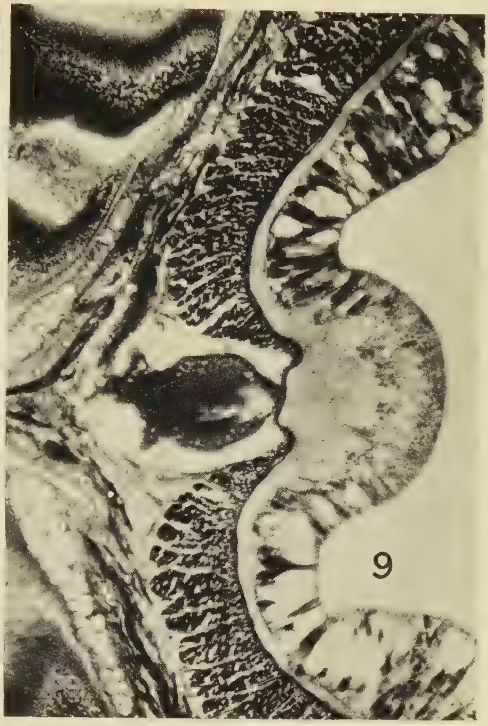
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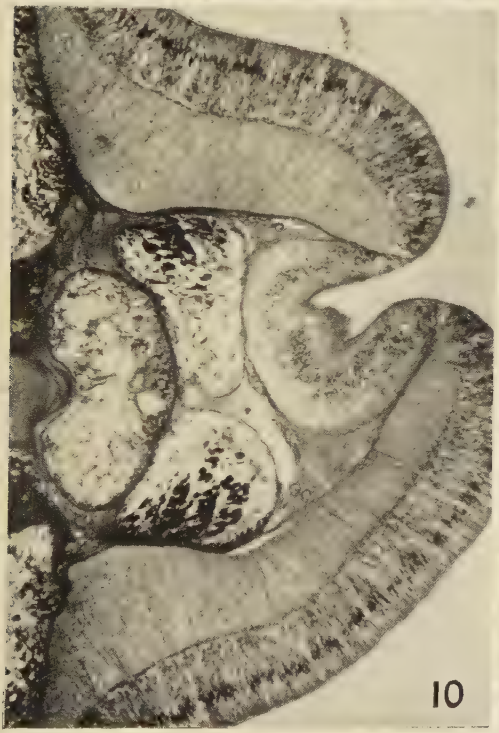
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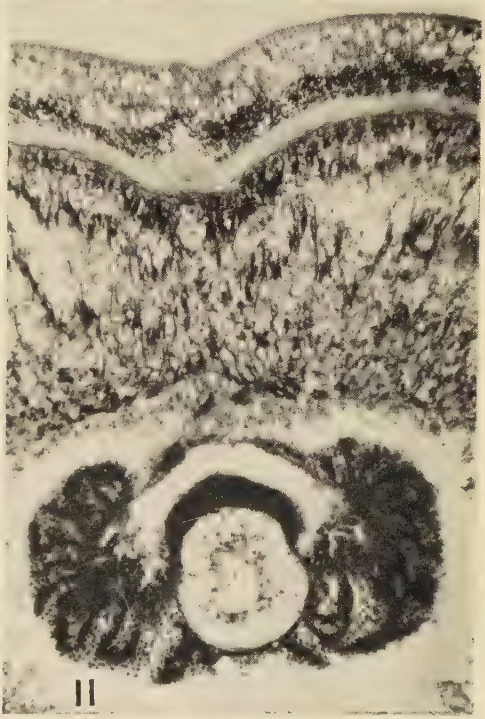
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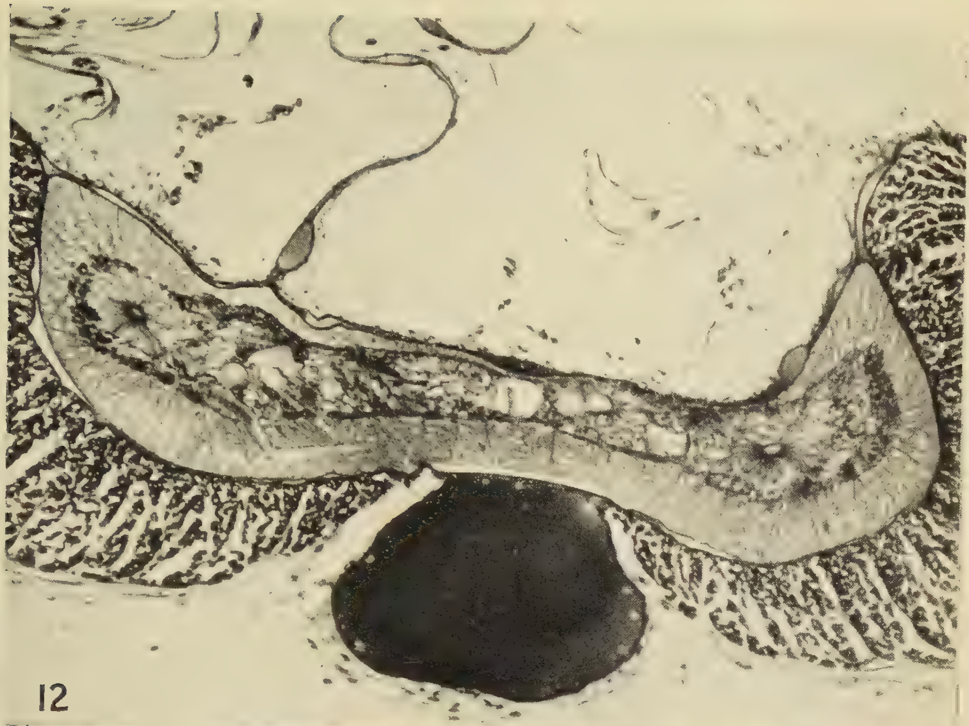

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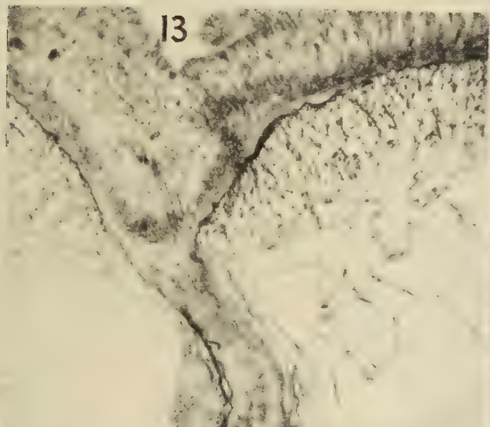

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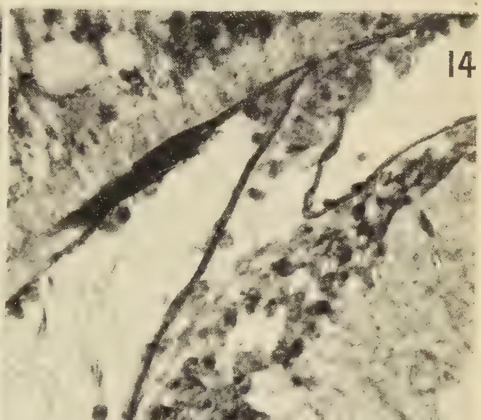

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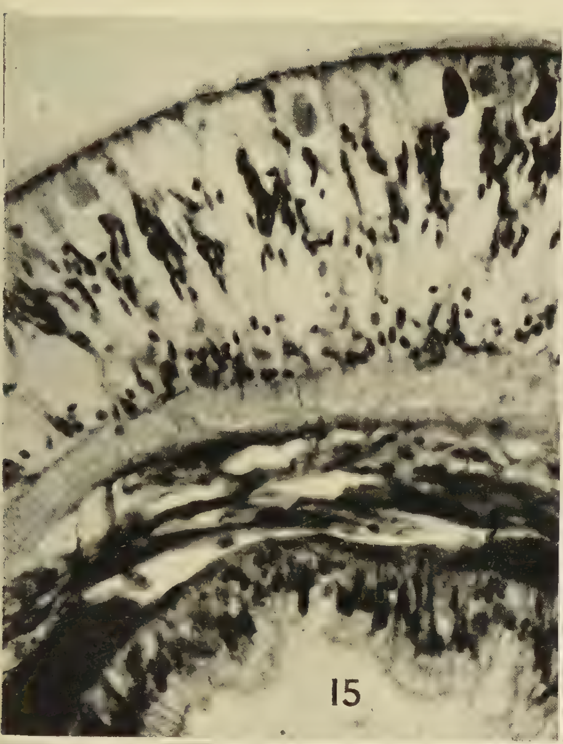

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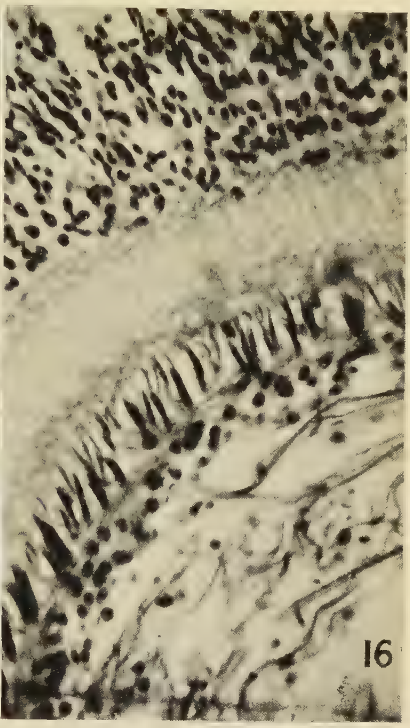

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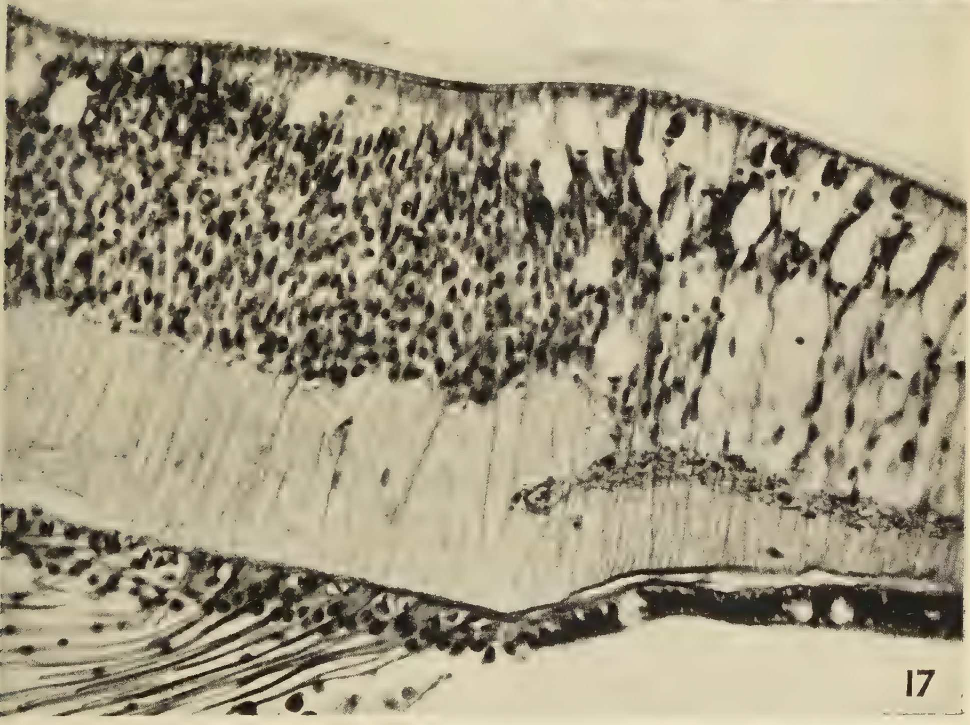

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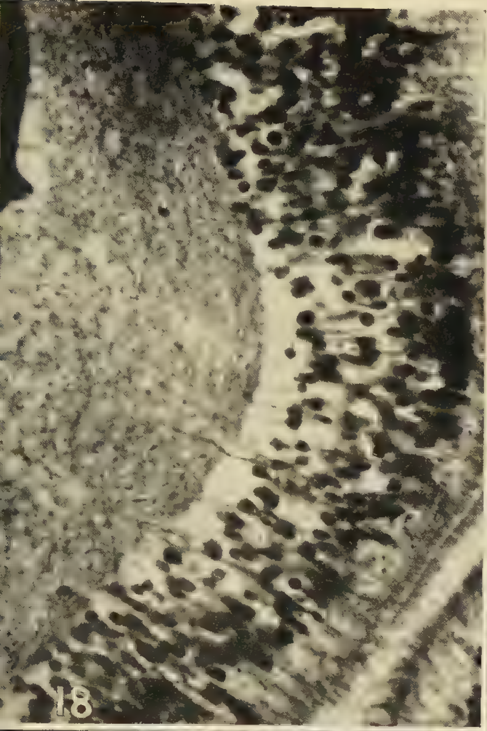

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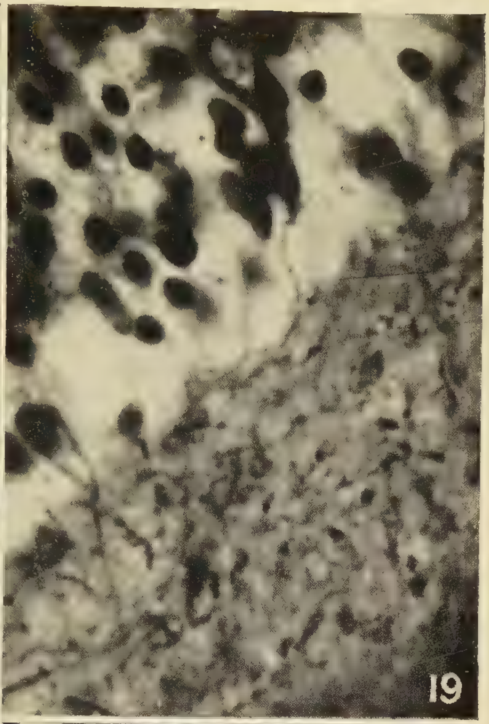

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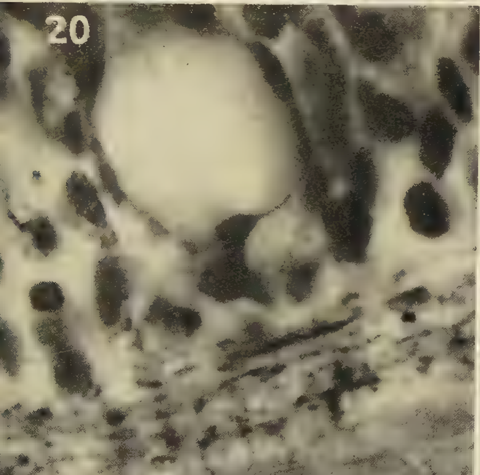

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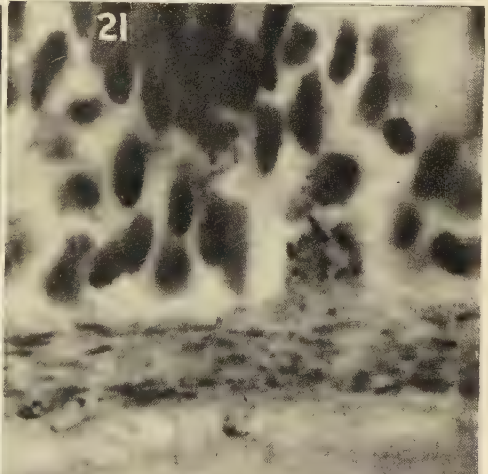

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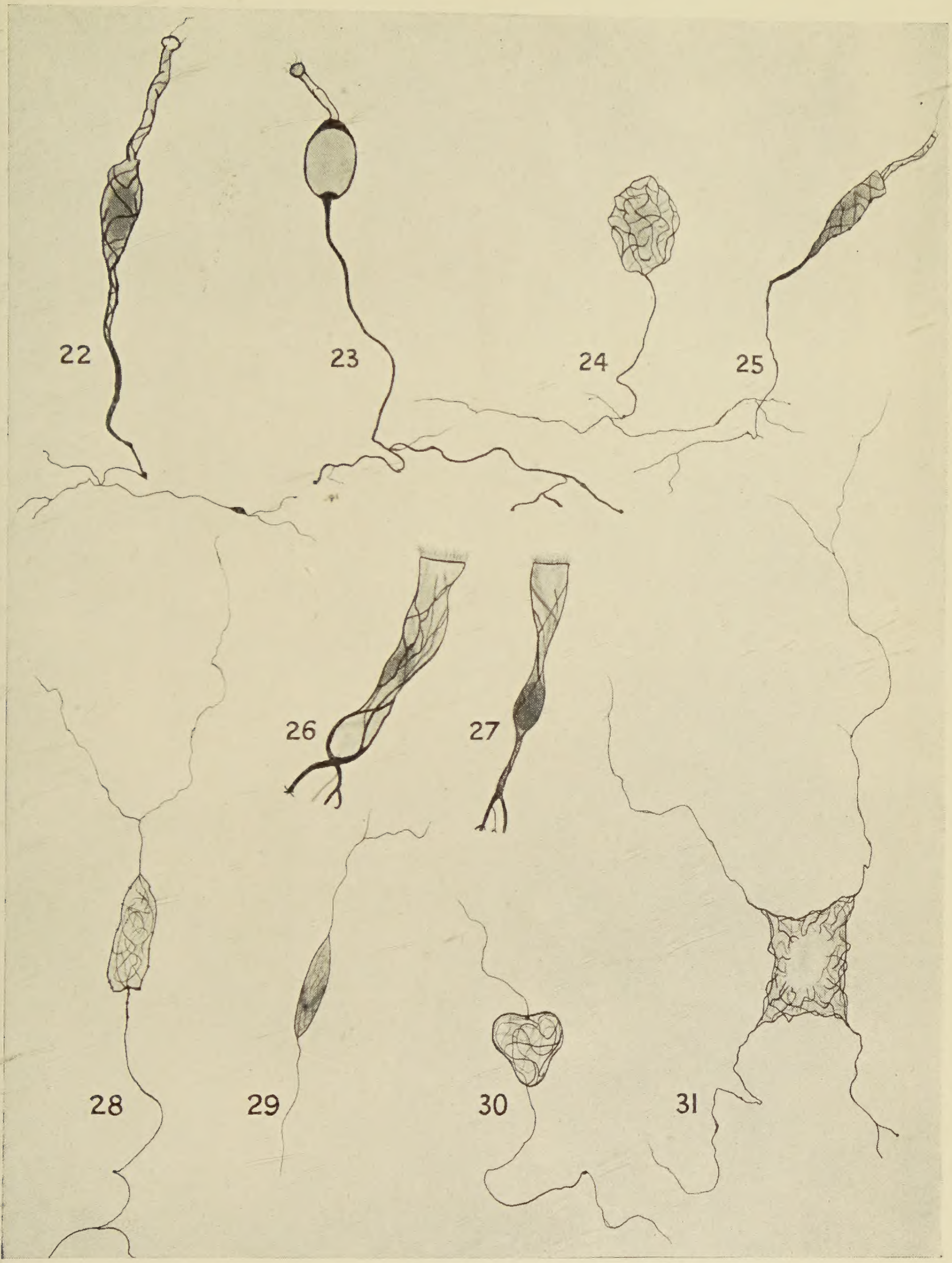

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